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(54) Title: POLYNUCLEOTIDES AND POLYPEPTIDES ENCODED THEREBY DISTANTLY HOMOLOGOUS TO HEPARANASE

(57) Abstract:

POLYNUCLEOTIDES AND POLYPEPTIDES ENCODED THEREBY  
DISTANTLY HOMOLOGOUS TO HEPARANASE

FIELD AND BACKGROUND OF THE INVENTION

5       The present invention relates to novel polynucleotides encoding polypeptides distantly homologous to heparanase, nucleic acid constructs including the polynucleotides, genetically modified cells expressing same, recombinant proteins encoded thereby and which may have heparanase or other glycosyl hydrolase activity, antibodies recognizing the recombinant  
10       proteins, oligonucleotides and oligonucleotide analogs derived from the polynucleotides and ribozymes including same.

Citation or identification of any reference in this application shall not be construed as an admission that such reference is available as prior art to the present invention.

15       *Glycosaminoglycans (GAGs)*

GAGs are polymers of repeated disaccharide units consisting of uronic acid and a hexosamine. Biosynthesis of GAGs except hyaluronic acid is initiated from a core protein. Proteoglycans may contain several GAG side chains from similar or different families. GAGs are synthesized  
20       as homopolymers which may subsequently be modified by N-deacetylation and N-sulfation, followed by C5-epimerization of glucuronic acid to iduronic acid and O-sulfation. The chemical composition of GAGs from various tissues varies highly.

The natural metabolism of GAGs in animals is carried out by  
25       hydrolysis. Generally, the GAGs are degraded in a two step procedure. First the proteoglycans are internalized in endosomes, where initial depolymerization of the GAG chain takes place. This step is mainly hydrolytic and yields oligosaccharides. Further degradation is carried out after fusion with lysosome, where desulfation and exolytic  
30       depolymerization to monosaccharides take place (42).

The only mammalian GAG degrading endolytic enzymes characterized so far are the hyaluronidases. The hyaluronidases are a family of 1-4 endoglucosaminidases that depolymerize hyaluronic acid and chondroitin sulfate. The cDNAs encoding sperm associated PH-20  
35       (Hyal3), and the lysosomal hyaluronidases Hyal 1 and Hyal2 were cloned and published (27). These enzymes share an overall homology of 40 % and have different tissue specificities, cellular localizations and PH optima.

Exolytic hydrolases are better characterized, among which are  $\beta$ -glucuronidase,  $\alpha$ -L-iduronidase, and  $\beta$ -N-acetylglucosaminidase. In addition to hydrolysis of the glycosidic bond of the polysaccharide chain, GAG degradation involves desulfation, which is catalyzed by several lysosomal sulfatases such as N-acetylgalactosamine-4-sulfatase, iduronate-2-sulfatase and heparin sulfamidase. Deficiency in any of lysosomal GAG degrading enzymes results in a lysosomal storage disease, mucopolysaccharidosis.

***Glycosyl hydrolases:***

Glycosyl hydrolases are a widespread group of enzymes that hydrolyze the o-glycosidic bond between two or more carbohydrates or between a carbohydrate and a noncarbohydrate moiety. The enzymatic hydrolysis of glycosidic bond occurs by using major one or two mechanisms leading to overall retention or inversion of the anomeric configuration. In both mechanisms catalysis involves two residues: a proton donor and a nucleophile. Glycosyl hydrolases have been classified into 58 families based on amino acid similarities. The glycosyl hydrolases from families 1, 2, 5, 10, 17, 30, 35, 39 and 42 act on a large variety of substrates, however, they all hydrolyze the glycosidic bond in a general acid catalysis mechanism, with retention of the anomeric configuration. The mechanism involves two glutamic acid residues, which are the proton donors and the nucleophile, with an asparagine always preceding the proton donor. Analyses of a set of known 3D structures from this group revealed that their catalytic domains, despite the low level of sequence identity, adopt a similar ( $\alpha/\beta$ ) 8 fold with the proton donor and the nucleophile located at the C-terminal ends of strands  $\beta$ 4 and  $\beta$ 7, respectively. Mutations in the functional conserved amino acids of lysosomal glycosyl hydrolases were identified in lysosomal storage diseases.

Lysosomal glycosyl hydrolases including  $\beta$ -glucuronidase,  $\beta$ -mannosidase,  $\beta$ -glucocerebrosidase,  $\beta$ -galactosidase and  $\alpha$ -L-iduronidase, are all exo-glycosyl hydrolases, belong to the GH-A clan and share a similar catalytic site. However, many endo-glucanases from various organisms, such as bacterial and fungal xylanases and cellulases share this catalytic domain (1).

***Heparan sulfate proteoglycans (HSPGs)***

HSPGs are ubiquitous macromolecules associated with the cell surface and extracellular matrix (ECM) of a wide range of cells of vertebrate and invertebrate tissues (3-7). The basic HSPG structure

consists of a protein core to which several linear heparan sulfate chains are covalently attached. The polysaccharide chains are typically composed of repeating hexuronic and D-glucosamine disaccharide units that are substituted to a varying extent with N- and O-linked sulfate moieties and N-linked acetyl groups (3-7). Studies on the involvement of ECM molecules in cell attachment, growth and differentiation revealed a central role of HSPGs in embryonic morphogenesis, angiogenesis, metastasis, neurite outgrowth and tissue repair (3-7). The heparan sulfate (HS) chains, which are unique in their ability to bind a multitude of proteins, ensure that a wide variety of effector molecules cling to the cell surface (6-8). HSPGs are also prominent components of blood vessels (5). In large vessels they are concentrated mostly in the intima and inner media, whereas in capillaries they are found mainly in the subendothelial basement membrane where they support proliferating and migrating endothelial cells and stabilize the structure of the capillary wall. The ability of HSPGs to interact with ECM macromolecules such as collagen, laminin and fibronectin, and with different attachment sites on plasma membranes suggests a key role for this proteoglycan in the self-assembly and insolubility of ECM components, as well as in cell adhesion and locomotion. Cleavage of HS may therefore result in disassembly of the subendothelial ECM and hence may play a decisive role in extravasation of normal and malignant blood-borne cells (9-11). HS catabolism is observed in inflammation, wound repair, diabetes, and cancer metastasis, suggesting that enzymes which degrade HS play important roles in pathologic processes.

#### ***Heparanase***

Heparanase is a glycosylated enzyme that is involved in the catabolism of certain glycosaminoglycans. It is an endoglucouronidase that cleaves heparan sulfate at specific intrachain sites (12-15). Interaction of T and B lymphocytes, platelets, granulocytes, macrophages and mast cells with the subendothelial extracellular matrix (ECM) is associated with degradation of heparan sulfate by heparanase activity (16). Connective tissue activating peptide III (CTAP), a c-chemokine, was found to have heparanase-like activity. Placenta heparanase acts as an adhesion molecule or as a degradative enzyme depending on the pH of the microenvironment (17).

Heparanase is released from intracellular compartments (e.g., lysosomes, specific granules) in response to various activation signals

(e.g., thrombin, calcium ionophores, immune complexes, antigens and mitogens), suggesting its regulated involvement in inflammation and cellular immunity responses (16).

It was also demonstrated that heparanase can be readily released  
5 from human neutrophils by 60 minutes incubation at 4 C in the absence of added stimuli (18).

Gelatinase, another ECM degrading enzyme which is found in tertiary granules of human neutrophils with heparanase, is secreted from the neutrophils in response to phorbol 12-myristate 13-acetate (PMA)  
10 treatment (19-20).

In contrast, various tumor cells appear to express and secrete heparanase in a constitutive manner in correlation with their metastatic potential (21).

Degradation of heparan sulfate by heparanase results in the release  
15 of heparin-binding growth factors, enzymes and plasma proteins that are sequestered by heparan sulfate in basement membranes, extracellular matrices and cell surfaces (22-23).

Heparanase activity has been described in a number of cell types including cultured skin fibroblasts, human neutrophils, activated rat T-lymphocytes, normal and neoplastic murine B-lymphocytes, human  
20 monocytes and human umbilical vein endothelial cells, SK hepatoma cells, human placenta and human platelets.

A procedure for purification of natural heparanase was reported for SK hepatoma cells and human placenta (U.S. Pat. No. 5,362,641) and for  
25 human platelets derived enzymes (62).

#### ***Cloning and expression of the heparanase gene***

A purified fraction of heparanase isolated from human hepatoma cells was subjected to tryptic digestion. Peptides were separated by high pressure liquid chromatography (HPLC) and micro sequenced. The  
30 sequence of one of the peptides was used to screen data bases for homology to the corresponding back translated DNA sequence. This procedure led to the identification of a clone containing an insert of 1020 base pairs (bp) which included an open reading frame of 963 bp followed by 27 bp of 3' untranslated region and a poly A tail. The new gene was  
35 designated *hpa*. Cloning of the missing 5' end of *hpa* was performed by Marathon RACE from placenta cDNA composite. The joined *hpa* cDNA (also referred to as *phpa*) fragment contained an open reading frame, which encodes a polypeptide of 543 amino acids with a calculated

molecular weight of 61,192 daltons (2). The cloning procedures are described in length in U.S. Pat. application Nos. 08/922,170, 09/109,386, and 09/258,892, the latter is a continuation-in-part of PCT/US98/17954, filed August 31, 1998, all of which are incorporated herein by reference.

5 The genomic locus which encodes heparanase spans about 40 kb. It is composed of 12 exons separated by 11 introns and is localized on human chromosome 4.

The ability of the *hpa* gene product to catalyze degradation of heparan sulfate (HS) *in vitro* was examined by expressing the entire open  
10 reading frame of *hpa* in High five and Sf21 insect cells, and the mammalian human 293 embryonic kidney cell line expression systems. Extracts of infected or transfected cells were assayed for heparanase catalytic activity. For this purpose, cell lysates were incubated with sulfate labeled, ECM-derived HSPG (peak I), followed by gel filtration analysis  
15 (Sepharose 6B) of the reaction mixture. While the substrate alone consisted of high molecular weight material, incubation of the HSPG substrate with lysates of cells infected or transfected with *hpa* containing vectors resulted in a complete conversion of the high molecular weight  
20 substrate into low molecular weight labeled heparan sulfate degradation fragments (see, for example, U.S. Pat. application No. 09/071,618, which is incorporated herein by reference.

In other experiments, it was demonstrated that the heparanase enzyme expressed by cells infected with a pF*hpa* virus is capable of degrading HS complexed to other macromolecular constituents (e.g.,  
25 fibronectin, laminin, collagen) present in a naturally produced intact ECM (see U.S. Pat. application No. 09/109,386, which is incorporated herein by reference), in a manner similar to that reported for highly metastatic tumor cells or activated cells of the immune system (7, 8).

30 ***Preferential expression of the hpa gene in human breast and hepatocellular carcinomas***

Semi-quantitative RT-PCR was applied to evaluate the expression of the *hpa* gene by human breast carcinoma cell lines exhibiting different degrees of metastasis. A marked increase in *hpa* gene expression is observed which correlates to metastatic capacity of non-metastatic MCF-7  
35 breast carcinoma, moderately metastatic MDA 231 and highly metastatic MDA 435 breast carcinoma cell lines. Significantly, the differential pattern of the *hpa* gene expression correlated with the pattern of heparanase activity.

Expression of the *hpa* gene in human breast carcinoma was demonstrated by *in situ* hybridization to archival paraffin embedded human breast tissue. Hybridization of the heparanase antisense riboprobe to invasive duct carcinoma tissue sections resulted in a massive positive staining localized specifically to the carcinoma cells. The *hpa* gene was also expressed in areas adjacent to the carcinoma showing fibrocystic changes. Normal breast tissue derived from reduction mammoplasty failed to express the *hpa* transcript. High expression of the *hpa* gene was also observed in tissue sections derived from human hepatocellular carcinoma specimens but not in normal adult liver tissue. Furthermore, tissue specimens derived from adenocarcinoma of the ovary, squamous cell carcinoma of the cervix and colon adenocarcinoma exhibited strong staining with the *hpa* RNA probe, as compared to a very low staining of the *hpa* mRNA in the respective non-malignant control tissues (2).

A preferential expression of heparanase in human tumors versus the corresponding normal tissues was also noted by immunohistochemical staining of paraffin embedded sections with monoclonal anti-heparanase antibodies. Positive cytoplasmic staining was found in neoplastic cells of the colon carcinoma and in dysplastic epithelial cells of a tubulovillous adenoma found in the same specimen while there was little or no staining of the normal looking colon epithelium located away from the carcinoma. Of particular significance was an intense immunostaining of colon adenocarcinoma cells that had metastasized into the liver, as compared to the surrounding normal liver tissue.

#### ***Latent and active forms of the heparanase protein***

The apparent molecular size of the recombinant enzyme produced in the baculovirus expression system was about 65 kDa. This heparanase polypeptide contains 6 potential N-glycosylation sites. Following deglycosylation by treatment with peptide N-glycosidase, the protein appeared as a 57 kDa band. This molecular weight corresponds to the deduced molecular mass (61,192 daltons) of the 543 amino acid polypeptide encoded by the full length *hpa* cDNA after cleavage of the predicted 3 kDa signal peptide. No further reduction in the apparent size of the N-deglycosylated protein was observed following concurrent O-glycosidase and neuraminidase treatment. Deglycosylation had no detectable effect on enzymatic activity.

Unlike the baculovirus enzyme, expression of the full length heparanase polypeptide in mammalian cells (e.g., 293 kidney cells, CHO)

yielded a major protein of about 50 kDa and a minor about 65 kDa protein in cell lysates. Preferential release of the about 65 kDa form into the culture medium was noted in some of the transfected CHO clones. Comparison of the enzymatic activity of the two forms, using a semi-quantitative gel filtration assay, revealed that the 50 kDa enzyme is about 100-fold more active than the 65 kDa form. A similar difference was observed when the specific activity of the recombinant 65 kDa baculovirus enzyme was compared to that of the 50 kDa heparanase preparations purified from human platelets, SK-hep-1 cells, or placenta. These results suggest that the 50 kDa protein is a mature processed form of a latent heparanase precursor. Amino terminal sequencing of the platelet heparanase indicated that cleavage occurs between amino acids glu<sup>157</sup>-lys<sup>158</sup>. As indicated by the hydropathic plot of heparanase, this site is located within a hydrophilic peak which is likely to be exposed and hence accessible to proteases.

#### *Involvement of Heparanase in Tumor Cell Invasion and Metastasis*

Circulating tumor cells arrested in the capillary beds often attach at or near the intercellular junctions between adjacent endothelial cells. Such attachment of the metastatic cells is followed by rupture of the junctions, retraction of the endothelial cell borders and migration through the breach in the endothelium toward the exposed underlying base membrane (BM) (24). Once located between endothelial cells and the BM, the invading cells must degrade the subendothelial glycoproteins and proteoglycans of the BM in order to migrate out of the vascular compartment. Several cellular enzymes (e.g., collagenase IV, plasminogen activator, cathepsin B, elastase, etc.) are thought to be involved in degradation of BM (25). Among these enzymes is heparanase that cleaves HS at specific intrachain sites (16, 11). Expression of a HS degrading heparanase was found to correlate with the metastatic potential of mouse lymphoma (26), fibrosarcoma and melanoma (21) cells. Moreover, elevated levels of heparanase were detected in sera from metastatic tumor bearing animals and melanoma patients (21) and in tumor biopsies of cancer patients (12).

The inhibitory effect of various non-anticoagulant species of heparin on heparanase was examined in view of their potential use in preventing extravasation of blood-borne cells. Treatment of experimental animals with heparanase inhibitors markedly reduced (> 90 %) the incidence of lung metastases induced by B16 melanoma, Lewis lung



carcinoma and mammary adenocarcinoma cells (12, 13, 28). Heparin fractions with high and low affinity to anti-thrombin III exhibited a comparable high anti-metastatic activity, indicating that the heparanase inhibiting activity of heparin, rather than its anticoagulant activity, plays a role in the anti-metastatic properties of the polysaccharide (12).

The direct role of heparanase in cancer metastasis was demonstrated by two experimental systems. The murine T-lymphoma cell line Eb has no detectable heparanase activity. Whether the introduction of the *hpa* gene into Eb cells would confer a metastatic behavior on these cells was investigated. To this purpose, Eb cells were transfected with a full length human *hpa* cDNA. Stable transfected cells showed high expression of the heparanase mRNA and enzyme activity. These *hpa* and mock transfected Eb cells were injected subcutaneously into DBA/2 mice and mice were tested for survival time and liver metastases. All mice (n=20) injected with mock transfected cells survived during the first 4 weeks of the experiment, while 50% mortality was observed in mice inoculated with Eb cells transfected with the *hpa* cDNA. The liver of mice inoculated with *hpa* transfected cells was infiltrated with numerous Eb lymphoma cells, as was evident both by macroscopic evaluation of the liver surface and microscopic examination of tissue sections. In contrast, metastatic lesions could not be detected by gross examination of the liver of mice inoculated with mock transfected control Eb cells. Few or no lymphoma cells were found to infiltrate the liver tissue. In a different model of tumor metastasis, transient transfection of the heparanase gene into low metastatic B16-F1 mouse melanoma cells followed by i.v. inoculation, resulted in a 4- to 5-fold increase in lung metastases.

Finally, heparanase externally adhered to B16-F1 melanoma cells increased the level of lung metastases in C57BL mice as compared to control mice (see U.S. Pat. application No. 09/260,037, entitled INTRODUCING A BIOLOGICAL MATERIAL INTO A PATIENT, which is a continuation in part of U.S. Pat. application No. 09/140,888, and is incorporated herein by reference.

***Possible involvement of heparanase in tumor angiogenesis***

Fibroblast growth factors are a family of structurally related polypeptides characterized by high affinity to heparin (29). They are highly mitogenic for vascular endothelial cells and are among the most potent inducers of neovascularization (29-30). Basic fibroblast growth factor (bFGF) has been extracted from a subendothelial ECM produced *in*

*vitro* (31) and from basement membranes of the cornea (32), suggesting that ECM may serve as a reservoir for bFGF. Immunohistochemical staining revealed the localization of bFGF in basement membranes of diverse tissues and blood vessels (23). Despite the ubiquitous presence of bFGF in normal tissues, endothelial cell proliferation in these tissues is usually very low, suggesting that bFGF is somehow sequestered from its site of action. Studies on the interaction of bFGF with ECM revealed that bFGF binds to HSPG in the ECM and can be released in an active form by HS degrading enzymes (33, 32, 34). It was demonstrated that heparanase activity expressed by platelets, mast cells, neutrophils, and lymphoma cells is involved in release of active bFGF from ECM and basement membranes (35), suggesting that heparanase activity may not only function in cell migration and invasion, but may also elicit an indirect neovascular response. These results suggest that the ECM HSPG provides a natural storage depot for bFGF and possibly other heparin-binding growth promoting factors (36,37). Displacement of bFGF from its storage within basement membranes and ECM may therefore provide a novel mechanism for induction of neovascularization in normal and pathological situations.

Recent studies indicate that heparin and HS are involved in binding of bFGF to high affinity cell surface receptors and in bFGF cell signaling (38, 39). Moreover, the size of HS required for optimal effect was similar to that of HS fragments released by heparanase (40). Similar results were obtained with vascular endothelial cells growth factor (VEGF) (41), suggesting the operation of a dual receptor mechanism involving HS in cell interaction with heparin-binding growth factors. It is therefore proposed that restriction of endothelial cell growth factors in ECM prevents their systemic action on the vascular endothelium, thus maintaining a very low rate of endothelial cells turnover and vessel growth. On the other hand, release of bFGF from storage in ECM as a complex with HS fragment, may elicit localized endothelial cell proliferation and neovascularization in processes such as wound healing, inflammation and tumor development (36,37).

***The involvement of heparanase in other physiological processes and its potential therapeutic applications***

Apart from its involvement in tumor cell metastasis, inflammation and autoimmunity, mammalian heparanase may be applied to modulate bioavailability of heparin-binding growth factors; cellular responses to heparin-binding growth factors (e.g., bFGF, VEGF) and cytokines (IL-8)

(44, 41); cell interaction with plasma lipoproteins (49); cellular susceptibility to certain viral and some bacterial and protozoa infections (45-47); and disintegration of amyloid plaques (48).

***Viral Infection:*** The presence of heparan sulfate on cell surfaces have been shown to be the principal requirement for the binding of Herpes Simplex (45) and Dengue (46) viruses to cells and for subsequent infection of the cells. Removal of the cell surface heparan sulfate by heparanase may therefore abolish virus infection. In fact, treatment of cells with bacterial heparitinase (degrading heparan sulfate) or heparinase (degrading heparan) reduced the binding of two related animal herpes viruses to cells and rendered the cells at least partially resistant to virus infection (45). There are some indications that the cell surface heparan sulfate is also involved in HIV infection (47).

***Neurodegenerative diseases:*** Heparan sulfate proteoglycans were identified in the prion protein amyloid plaques of Genstmann-Straussler Syndrome, Creutzfeldt-Jakob disease and Scrape (48). Heparanase may disintegrate these amyloid plaques which are also thought to play a role in the pathogenesis of Alzheimer's disease.

***Restenosis and Atherosclerosis:*** Proliferation of arterial smooth muscle cells (SMCs) in response to endothelial injury and accumulation of cholesterol rich lipoproteins are basic events in the pathogenesis of atherosclerosis and restenosis (50). Apart from its involvement in SMC proliferation as a low affinity receptor for heparin-binding growth factors, HS is also involved in lipoprotein binding, retention and uptake (51). It was demonstrated that HSPG and lipoprotein lipase participate in a novel catabolic pathway that may allow substantial cellular and interstitial accumulation of cholesterol rich lipoproteins (49). The latter pathway is expected to be highly atherogenic by promoting accumulation of apoB and apoE rich lipoproteins (e.g., LDL, VLDL, chylomicrons), independent of feed back inhibition by the cellular cholesterol content. Removal of SMC HS by heparanase is therefore expected to inhibit both SMC proliferation and lipid accumulation and thus may halt the progression of restenosis and atherosclerosis.

***Pulmonary diseases:*** The data obtained from the literature suggests a possible role for GAGs degrading enzymes, such as, but not limited to, heparanases, connective tissue activating peptide, heparinases, hyaluronidases, sulfatases and chondroitinases, in reducing the viscosity of sinuses and airway

secretions with associated implications on curtailing the rate of infection and inflammation. The sputum from CF patients contains at least 3 % GAGs, thus contributing to its volume and viscous properties. Recombinant heparanase has been shown to reduce viscosity of sputum of CF patients (see, U.S. Pat. application No. 09/046,475).

In summary, heparanase may thus prove useful for conditions such as wound healing, angiogenesis, restenosis, atherosclerosis, inflammation, neurodegenerative diseases and viral infections. Mammalian heparanase can be used to neutralize plasma heparin, as a potential replacement of protamine. Anti-heparanase antibodies may be applied for immunodetection and diagnosis of micrometastases, autoimmune lesions and renal failure in biopsy specimens, plasma samples, and body fluids.

There is thus a widely recognized need for, and it would be highly advantageous to have, additional molecules with glycosyl hydrolase activity, because such molecules may exhibit greater specific activity toward certain substrates or different substrate specificity than the known heparanase.

#### SUMMARY OF THE INVENTION

According to one aspect of the present invention there is provided an isolated nucleic acid comprising a polynucleotide hybridizable with SEQ ID NOs:1, 4, 6 or portions thereof at 68 °C in 6 x SSC, 1 % SDS, 5 x Denharts, 10 % dextran sulfate, 100 µg/ml salmon sperm DNA, and <sup>32</sup>p labeled probe and wash at 68 °C with 3 x SSC and 0.1 % SDS.

According to another aspect of the present invention there is provided an isolated nucleic acid comprising a polynucleotide hybridizable with SEQ ID NOs:1, 4, 6 or portions thereof at 68 °C in 6 x SSC, 1 % SDS, 5 x Denharts, 10 % dextran sulfate, 100 µg/ml salmon sperm DNA, and <sup>32</sup>p labeled probe and wash at 68 °C with 1 x SSC and 0.1 % SDS.

According to still another aspect of the present invention there is provided an isolated nucleic acid comprising a polynucleotide hybridizable with SEQ ID NOs:1, 4, 6 or portions thereof at 68 °C in 6 x SSC, 1 % SDS, 5 x Denharts, 10 % dextran sulfate, 100 µg/ml salmon sperm DNA, and <sup>32</sup>p labeled probe and wash at 68 °C with 0.1 x SSC and 0.1 % SDS.

According to yet another aspect of the present invention there is provided an isolated nucleic acid comprising a polynucleotide at least 60 % identical with SEQ ID NOs:1, 4, 6 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software

package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

According to still another aspect of the present invention there is provided an isolated nucleic acid comprising a polynucleotide encoding a polypeptide being at least 60 % homologous with SEQ ID NOs:3, 5, 7 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

According to further features in preferred embodiments of the invention described below, the polynucleotide is as set forth in SEQ ID NOs:1, 4, 6 or portions thereof.

According to an additional aspect of the present invention there is provided a recombinant protein comprising a polypeptide encoded by the polynucleotides herein described.

According to yet an additional aspect of the present invention there is provided a recombinant protein comprising a polypeptide at least 60 % homologous with SEQ ID NOs:3, 5, 7 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

According to further features in preferred embodiments of the invention described below, the polypeptide is as set forth in SEQ ID NOs:3, 5, 7 or portions thereof.

According to still an additional aspect of the present invention there is provided a nucleic acid construct comprising the isolated nucleic acid herein described.

According to a further aspect of the present invention there is provided a nucleic acid construct comprising a polynucleotide encoding the recombinant protein herein described.

According to still a further aspect of the present invention there is provided a host cell comprising a polynucleotide or construct and/or expressing a recombinant protein as herein described.

According to yet a further aspect of the present invention there is provided an antisense oligonucleotide or nucleic acid construct comprising a polynucleotide or a polynucleotide analog of at least 10 bases being

hybridizable *in vivo*, under physiological conditions, with (i) a portion of a polynucleotide strand encoding a polypeptide at least 60 % homologous with SEQ ID NOs:3, 5, 7 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3); or (ii) a portion of a polynucleotide strand at least 60 % identical with SEQ ID NOs:1, 4, 6 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

According to another aspect of the present invention there is provided a ribozyme comprising the antisense oligonucleotide herein described and a ribozyme sequence.

The present invention provides polynucleotides and polypeptides belonging to a class of asp-glu glycosyl hydrolases of the GH-A clan, probably, based on homology to heparanase, GAG degrading enzymes.

#### BRIEF DESCRIPTION OF THE DRAWINGS

The invention is herein described, by way of example only, with reference to the accompanying drawings, wherein:

FIG. 1 shows the nucleotide sequence (SEQ ID NOs:1-2) and the deduced amino acid sequence (SEQ ID NOs:2-3) of *hnhp1*;

FIG. 2 is a comparison of the deduced amino acid sequences of *hnhp1* (SEQ ID NOs:2-3) and of heparanase (SEQ ID NO:9). Comparison was performed using the Gap program of the GCG package (gap creation penalty - 50, gap extension penalty - 3);

FIG. 3 illustrates variability of *hnhp1* transcripts. *Hnhp1* was amplified from placenta and from testis marathon ready cDNA libraries, using the gene specific primers pn9-312u (SEQ ID NO:14) and hn11-230 (SEQ ID NO:11);

FIG. 4 shows a zoo blot. Ten micrograms of genomic DNA from various species were digested with *EcoRI* and separated on 0.7 % agarose - TBE gel. Following electrophoresis, the gel was treated with HCl and then with NaOH and the DNA fragments were downward transferred to a nylon membrane (Hybond N+, Amersham) with 0.4 N NaOH. The membrane was hybridized with a 1.7 Kb DNA probe that contained the *hnhp1* cDNA (clone pn9). Lane order: H - Human; M - Mouse; Rt - Rat; P

- Pig; Cw - Cow; Hr - Horse; S - Sheep; Rb - Rabbit; D - Dog; Ch - Chicken; F - Fish. Size markers (Lambda *Bst*II) are shown on the left;

FIG. 5 illustrates cross hybridization between *hpa* and *hnhp1*. *Hpa* was amplified by PCR from marathon ready placenta cDNA library. *Hnhp1* was amplified from testis marathon ready cDNA library. PCR products were run on agarose gel in duplicates and transferred to a nylon membrane. One membrane was probed with <sup>32</sup>p labeled *hpa* cDNA and the other with *hnhp1*, clone pn9.

FIG. 6 is a comparison of the hydropathic profiles of heparanase and *hnhp1*. The curves were calculated according to the Kyte and Doolittle method over a window of 17 amino acids.

FIG. 7 shows a Western blot analysis of recombinant *hnhp1* expressed in human embryonal kidney 293 cells. A - control heparanase-FLAG precursor, B-D - 293 cells transfected with a control pSI vector (B), pSI-pn6 (C) and pSI-pn9 (D). Cell extracts were separated by SDS-PAGE, transferred onto Immobilon-P nylon membrane (Millipore). Membrane was incubated with anti-FLAG Flag antibody 1:1000 (Kodak anti Flag M2 cat: IB13025).

## DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention is of novel polynucleotides encoding polypeptides distantly homologous to heparanase, nucleic acid constructs including the polynucleotides, genetically modified cells expressing same, recombinant proteins encoded thereby and which may have heparanase or other glycosyl hydrolase activity, antibodies recognizing the recombinant proteins, oligonucleotides and oligonucleotide analogs derived from the polynucleotides and ribozymes including same.

The principles and operation of the present invention may be better understood with reference to the drawings and accompanying descriptions.

Before explaining at least one embodiment of the invention in detail, it is to be understood that the invention is not limited in its application to the details of construction and the arrangement of the components set forth in the following description or illustrated in the drawings. The invention is capable of other embodiments or of being practiced or carried out in various ways. Also, it is to be understood that the phraseology and terminology employed herein is for the purpose of description and should not be regarded as limiting.

While reducing the present invention to practice the human EST database was screened for homologous sequences using the entire amino acid sequence of human heparanase (SEQ ID NO:9). A distantly homologous fragment was pooled out, accession number AI222323, IMAGE clone number 1843155 from Soares\_NFL\_T\_GBC\_S1 Homo Sapiens cDNA library prepared from testis B-cells and fetal lungs. The clone contained an insert of 560 bp (SEQ ID NO:23) of which the 3' region was homologous to the human *hpa* gene encoding human heparanase. Primers derived from the newly identified clone were used to isolate several cDNAs including several open reading frames which reflect in frame alternative splicing, the longest of which, pn6, appears in Figure 1 (SEQ ID NOs:1, 2 and 3) is 2060 nucleotide long and it contains an open reading frame of 1776 nucleotides, which encodes a polypeptide of 592 amino acids, with a calculated molecular weight of 66.5 kDa. The newly cloned gene was designated *hnhp1*. Two shorter forms, pn9 and pn5 and their deduced amino acid sequences are set forth in SEQ ID NOs:4 and 6 and SEQ ID NO:5 and 7, respectively, and are further described in the Examples section that follows. Comparison between the amino acid sequence of *hnhp1* and heparanase is shown in Figure 3. The homology between the two proteins is 52.8 % or 55.3 %, depending on the software employed. No cross hybridization was detected between *hpa* and *hnhp1*, even under very moderate wash conditions (Figure 5). Zoo blot analysis demonstrated that the *hnhp1* gene and other related genes, perhaps forming a new gene family, are present in genomes of other organisms including mammals and avians. The chromosome localization of *hnhp1* was determined using G3 radiation hybrid panel to be on human chromosome 10, next to the marker SHGC-57721. The results also indicated a possibility of a second copy of the gene or of a related gene. The *hnhp1* gene is expressed in low levels in lymph nodes, spleen, colon and ovary; in slightly higher levels in prostate and small intestine; and in yet more pronounced level in testis. No expression was detected under the assay employed in bone marrow, liver, thymus, tonsil or leukocytes. Screening of the mouse EST database with the amino acid sequence of heparanase as well as of *hnhp1* pooled out a mouse EST clone (clone 1378452 accession number AI019269 from mouse thymus, SEQ ID NO:8). However, this clone includes two frame shift mutations which hamper its open reading frame.



The overall homology between the amino acid sequence of *hnhp1* and heparanase suggest that these two proteins share similar function. The homology between the two proteins is concentrated at several regions. These may represent functional domains of the protein. The variability  
5 may suggest potential difference in substrate recognition, cellular localization and parameters of activity.

Despite the lack of an overall homology between the heparanase and other glycosyl hydrolases, the amino acid couple asp-glu (NE, SEQ ID NO:13), which is characteristic of the proton donor of glycosyl hydrolyses  
10 of the GH-A clan, was found at positions 224, 225 of heparanase. As in other clan members, this NE couple is located at the end of a  $\beta$  strand. As shown in Figure 2, the region surrounding the NE couple is conserved in the predicted amino acid sequence of *hnhp1*. This suggests that *hnhp1* product is a glycosyl hydrolase. This definition may include any  
15 polysaccharide degrading enzyme, either exo or endo glycosidase and based on the similarity to heparanase it is likely that it encodes a GAG degrading enzyme.

In addition, superimposition of the hydropathic profiles of heparanase and *hnhp1* (Figure 6) indicates an overlapping pattern along  
20 the proteins. The amino acid sequence characteristic of glycosyl hydrolases is located within a hydrophilic peak and at the same position in the aligned proteins. A remarkable difference in the hydropathic pattern is noticed around amino acids 157, 158 of heparanase, which constitute the processing site of the enzyme. While in heparanase, this site is located at  
25 the tip of a hydrophilic peak, the equivalent region of *hnhp1* is rather not hydrophilic. The peak around amino acid 110 of heparanase appears also, around amino acid 130 of *hnhp1*. Cleavage of heparanase at this region was shown to result in enzyme activation. The equivalent region of *hnhp1* might be a potential processing site.

30 Heparanase has a potential signal peptide at the N-terminus of the 67 kDa form. The homology between the two proteins is low at the N-termini and no signal peptide was identified in *hnhp1* polypeptide.

According to one aspect of the present invention there is provided an isolated nucleic acid comprising a polynucleotide hybridizable with  
35 SEQ ID NOs:1, 4, 6 or portions thereof at 68 °C in 6 x SSC, 1 % SDS, 5 x Denharts, 10 % dextran sulfate, 100  $\mu$ g/ml salmon sperm DNA, and  $^{32}$ p labeled probe and wash at 68 °C with 3 x SSC, 1 x SSC or 0.1 x SSC and 0.1 % SDS.

As used herein in the specification and in the claims section that follows, the term "portion" or "portions" refer to a consecutive stretch of nucleic or amino acids. Such a portion may include, for example, at least 90 nucleotides (equivalent to at least 30 amino acids), at least 120  
5 nucleotides (equivalent to at least 40 amino acids), at least 150 nucleotides (equivalent to at least 50 amino acids), at least 180 nucleotides (equivalent to at least 60 amino acids), at least 210 nucleotides (equivalent to at least 70 amino acids), at least 300 nucleotides (equivalent to at least 100 amino acids), at least 600 nucleotides (equivalent to at least 200 amino acids), at least 900 nucleotides (equivalent to at least 300 amino acids), at least  
10 1,200 nucleotides (equivalent to at least 400 amino acids), at least 1,500 nucleotides (equivalent to at least 500 amino acids), or more.

According to another aspect of the present invention there is provided an isolated nucleic acid comprising a polynucleotide at least 60  
15 %, preferably at least 65 %, more preferably at least 70 %, still preferably at least 75 %, yet preferably at least 80 %, more preferably at least 85 %, more preferably at least 90 %, most preferably at least 95 % - 100 %, identical with SEQ ID NOs:1, 4, 6 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of  
20 Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

According to still another aspect of the present invention there is provided an isolated nucleic acid comprising a polynucleotide encoding a polypeptide being at least 60 %, preferably at least 65 %, more preferably  
25 at least 70 %, still preferably at least 75 %, yet preferably at least 80 %, more preferably at least 85 %, more preferably at least 90 %, most preferably at least 95 % - 100 %, homologous with SEQ ID NOs:3, 5, 7 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer  
30 Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

As used herein in the specification and in the claims section that follows, the term "homologous" refers to identical + similar.

According to an additional aspect of the present invention there is  
35 provided a recombinant protein comprising a polypeptide encoded by the polynucleotides herein described.

The nucleic acid according to the present invention can be a complementary polynucleotide sequence, genomic polynucleotide sequence or a composite polynucleotide sequence.

As used herein the phrase "complementary polynucleotide sequence" includes sequences which originally result from reverse transcription of messenger RNA using a reverse transcriptase or any other RNA dependent DNA polymerase. Such sequences can be subsequently amplified *in vivo* or *in vitro* using a DNA dependent DNA polymerase.

As used herein the phrase "genomic polynucleotide sequence" includes sequences which originally derive from a chromosome and reflect a contiguous portion of a chromosome.

As used herein the phrase "composite polynucleotide sequence" includes sequences which are at least partially complementary and at least partially genomic. A composite sequence can include some exonal sequences required to encode a polypeptide, as well as some intronic sequences interposing therebetween. The intronic sequences can be of any source, including of other genes, and typically will include conserved splicing signal sequences. Such intronic sequences may further include cis acting expression regulatory elements.

Thus, this aspect of the present invention encompasses (i) polynucleotides as set forth in SEQ ID NOs:1, 4 and 6; (ii) fragments or portions thereof; (iii) sequences hybridizable therewith; (iv) sequences homologous thereto; (v) genomic and composite sequences corresponding thereto; (vi) sequences encoding similar polypeptides with different codon usage; and (vii) altered sequences characterized by mutations, such as deletion, insertion or substitution of one or more nucleotides, either naturally occurring or man induced, either randomly or in a targeted fashion.

According to yet an additional aspect of the present invention there is provided a recombinant protein comprising a polypeptide at least 60 %, preferably at least 65 %, more preferably at least 70 %, still preferably at least 75 %, yet preferably at least 80 %, more preferably at least 85 %, more preferably at least 90 %, most preferably at least 95 % - 100 %, homologous with SEQ ID NOs:3, 5, 7 or portions thereof, as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

According to still an additional aspect of the present invention there is provided a nucleic acid construct comprising the isolated nucleic acid herein described.

According to a preferred embodiment of the present invention the  
5 nucleic acid construct further comprising a promoter for regulating the  
expression of the isolated nucleic acid in a sense or antisense orientation.  
Such promoters are known to be *cis*-acting sequence elements required for  
transcription as they serve to bind DNA dependent RNA polymerase  
which transcribes sequences present downstream thereof. Such down  
10 stream sequences can be in either one of two possible orientations to result  
in the transcription of sense RNA which is translatable by the ribozyme  
machinery or antisense RNA which typically does not contain translatable  
sequences, yet can duplex or triplex with endogenous sequences, either  
mRNA or chromosomal DNA and hamper gene expression, all as further  
15 detailed hereinunder.

While the isolated nucleic acid described herein is an essential  
element of the invention, it is modular and can be used in different  
contexts. The promoter of choice that is used in conjunction with this  
invention is of secondary importance, and will comprise any suitable  
20 promoter. It will be appreciated by one skilled in the art, however, that it  
is necessary to make sure that the transcription start site(s) will be located  
upstream of an open reading frame. In a preferred embodiment of the  
present invention, the promoter that is selected comprises an element that  
is active in the particular host cells of interest. These elements may be  
25 selected from transcriptional regulators that activate the transcription of  
genes essential for the survival of these cells in conditions of stress or  
starvation, including, but not limited to, the heat shock proteins.

A construct according to the present invention preferably further  
includes an appropriate selectable marker. In a more preferred  
30 embodiment according to the present invention the construct further  
includes an origin of replication. In another most preferred embodiment  
according to the present invention the construct is a shuttle vector, which  
can propagate both in *E. coli* (wherein the construct comprises an  
appropriate selectable marker and origin of replication) and be compatible  
35 for propagation in cells, or integration in the genome, of an organism of  
choice. The construct according to this aspect of the present invention can  
be, for example, a plasmid, a bacmid, a phagemid, a cosmid, a phage, a  
virus or an artificial chromosome.

Alternatively, the nucleic acid construct according to this aspect of the present invention further includes a positive and a negative selection markers and may therefore be employed for selecting for homologous recombination events, including, but not limited to, homologous recombination employed in knock-in and knock-out procedures. One ordinarily skilled in the art can readily design a knock-out or knock-in constructs including both positive and negative selection genes for efficiently selecting transfected embryonic stem cells that underwent a homologous recombination event with the construct. Such cells can be introduced into developing embryos to generate chimeras, the offspring thereof can be tested for carrying the knock-out or knock-in constructs. Knock-out and/or knock-in constructs according to the present invention can be used to further investigate the functionality of the new gene. Such constructs can also be used in somatic and/or germ cells gene therapy to destroy activity of a defective, gain of function allele or to replace the lack of activity of a silent allele in an organism, thereby to down or upregulate activity, as required. Further detail relating to the construction and use of knock-out and knock-in constructs can be found in Fukushige, S. and Ikeda, J.E.: Trapping of mammalian promoters by Cre-lox site-specific recombination. *DNA Res* 3 (1996) 73-80; Bedell, M.A., Jenkins, N.A. and Copeland, N.G.: Mouse models of human disease. Part I: Techniques and resources for genetic analysis in mice. *Genes and Development* 11 (1997) 1-11; Bermingham, J.J., Scherer, S.S., O'Connell, S., Arroyo, E., Kalla, K.A., Powell, F.L. and Rosenfeld, M.G.: Tst-1/Oct-6/SCIP regulates a unique step in peripheral myelination and is required for normal respiration. *Genes Dev* 10 (1996) 1751-62, which are incorporated herein by reference.

According to yet another aspect of the present invention there is provided a host cell or animal comprising a nucleic acid construct or a portion thereof as described herein. Methods of transforming host cells, both prokaryotes and eukaryotes, and organisms with nucleic acid constructs and selection of transformants (e.g., transformed cells or transgenic animals) are well known to those of skills in the art. In addition, once transfected, such cells and organisms can be designed to direct the production of ample amounts of a recombinant protein which can then be purified by known methods, including, but not limited to, various chromatography and gel electrophoresis methods. Such a purified recombinant protein can serve for elicitation of antibodies as further

detailed hereinunder. Methods of transformation of cells and organism are described in detail in reference 43, whereas methods of recombinant protein purification are described in detail in reference 52, both are incorporated herein by reference.

5 According to still another aspect of the present invention there is provided an oligonucleotide of at least 17, at least 18, at least 19, at least 20, at least 22, at least 25, at least 30 or at least 40, bases specifically hybridizable with the isolated nucleic acid described herein.

Hybridization of shorter nucleic acids (below 200 bp in length, e.g.  
10 17-40 bp in length) is effected by stringent, moderate or mild hybridization, wherein stringent hybridization is effected by a hybridization solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 µg/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization  
15 temperature of 1 - 1.5 °C below the  $T_m$ , final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the  $T_m$ ; moderate hybridization is effected by a hybridization solution of 6 x SSC and 0.1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 µg/ml  
20 denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 2 - 2.5 °C below the  $T_m$ , final wash solution of 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS at 1 - 1.5 °C below the  $T_m$ , final wash solution of 6 x SSC, and final wash at 22 °C; whereas mild hybridization is effected by a hybridization  
25 solution of 6 x SSC and 1 % SDS or 3 M TMACI, 0.01 M sodium phosphate (pH 6.8), 1 mM EDTA (pH 7.6), 0.5 % SDS, 100 µg/ml denatured salmon sperm DNA and 0.1 % nonfat dried milk, hybridization temperature of 37 °C, final wash solution of 6 x SSC and final wash at 22 °C.

30 According to an additional aspect of the present invention there is provided a pair of oligonucleotides each independently of at least 17, at least 18, at least 19, at least 20, at least 22, at least 25, at least 30 or at least 40 bases specifically hybridizable with the isolated nucleic acid described herein in an opposite orientation so as to direct exponential  
35 amplification of a portion thereof in a nucleic acid amplification reaction, such as a polymerase chain reaction. The polymerase chain reaction and other nucleic acid amplification reactions are well known in the art and require no further description herein. The pair of oligonucleotides

according to this aspect of the present invention are preferably selected to have compatible melting temperatures ( $T_m$ ), e.g., melting temperatures which differ by less than that 7 °C, preferably less than 5 °C, more preferably less than 4 °C, most preferably less than 3 °C, ideally between 3 °C and zero °C. Consequently, according to yet an additional aspect of the present invention there is provided a nucleic acid amplification product obtained using the pair of primers described herein. Such a nucleic acid amplification product can be isolated by gel electrophoresis or any other size based separation technique. Alternatively, such a nucleic acid amplification product can be isolated by affinity separation, either strandness affinity or sequence affinity. In addition, once isolated, such a product can be further genetically manipulated by restriction, ligation and the like, to serve any one of a plurality of applications associated with up and/or down regulation of activity.

According to still an additional aspect of the present invention there is provided an antisense oligonucleotide comprising a polynucleotide or a polynucleotide analog of at least 10 bases, preferably between 10 and 15, more preferably between 50 and 20 bases, most preferably, at least 17, at least 18, at least 19, at least 20, at least 22, at least 25, at least 30 or at least 40 bases being hybridizable *in vivo*, under physiological conditions, with (i) a portion of a polynucleotide strand encoding a polypeptide at least 60 %, preferably at least 65 %, more preferably at least 70 %, still preferably at least 75 %, yet preferably at least 80 %, more preferably at least 85 %, more preferably at least 90 %, most preferably at least 95 % - 100 % homologous to SEQ ID NOs:3, 5, 7 or portions thereof as determined using the as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3); or (ii) a portion of a polynucleotide strand at least 60 %, preferably at least 65 %, more preferably at least 70 %, still preferably at least 75 %, yet preferably at least 80 %, more preferably at least 85 %, more preferably at least 90 %, most preferably at least 95 % - 100 % identical with SEQ ID NOs:1, 4, 6 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 12, gap extension penalty - 4).

Such antisense oligonucleotides can be used to downregulate gene expression as further detailed hereinunder. Such an antisense oligonucleotide is readily synthesizable using solid phase oligonucleotide synthesis.

- 5        The ability of chemically synthesizing oligonucleotides and analogs thereof having a selected predetermined sequence offers means for down modulating gene expression. Three types of gene expression modulation strategies may be considered.

- 10        At the transcription level, antisense or sense oligonucleotides or analogs that bind to the genomic DNA by strand displacement or the formation of a triple helix, may prevent transcription. At the transcript level, antisense oligonucleotides or analogs that bind target mRNA molecules lead to the enzymatic cleavage of the hybrid by intracellular RNase H. In this case, by hybridizing to the targeted mRNA, the  
15        oligonucleotides or oligonucleotide analogs provide a duplex hybrid recognized and destroyed by the RNase H enzyme. Alternatively, such hybrid formation may lead to interference with correct splicing. As a result, in both cases, the number of the target mRNA intact transcripts ready for translation is reduced or eliminated. At the translation level,  
20        antisense oligonucleotides or analogs that bind target mRNA molecules prevent, by steric hindrance, binding of essential translation factors (ribosomes), to the target mRNA, a phenomenon known in the art as hybridization arrest, disabling the translation of such mRNAs.

- 25        Thus, antisense sequences, which as described hereinabove may arrest the expression of any endogenous and/or exogenous gene depending on their specific sequence, attracted much attention by scientists and pharmacologists who were devoted at developing the antisense approach into a new pharmacological tool.

- 30        For example, several antisense oligonucleotides have been shown to arrest hematopoietic cell proliferation, growth, entry into the S phase of the cell cycle, reduced survival and prevent receptor mediated responses.

- 35        For efficient *in vivo* inhibition of gene expression using antisense oligonucleotides or analogs, the oligonucleotides or analogs must fulfill the following requirements (i) sufficient specificity in binding to the target sequence; (ii) solubility in water; (iii) stability against intra- and extracellular nucleases; (iv) capability of penetration through the cell membrane; and (v) when used to treat an organism, low toxicity.



Unmodified oligonucleotides are typically impractical for use as antisense sequences since they have short *in vivo* half-lives, during which they are degraded rapidly by nucleases. Furthermore, they are difficult to prepare in more than milligram quantities. In addition, such  
5 oligonucleotides are poor cell membrane penetrators.

Thus it is apparent that in order to meet all the above listed requirements, oligonucleotide analogs need to be devised in a suitable manner. Therefore, an extensive search for modified oligonucleotides has been initiated.

10 For example, problems arising in connection with double-stranded DNA (dsDNA) recognition through triple helix formation have been diminished by a clever "switch back" chemical linking, whereby a sequence of polypurine on one strand is recognized, and by "switching back", a homopurine sequence on the other strand can be recognized.  
15 Also, good helix formation has been obtained by using artificial bases, thereby improving binding conditions with regard to ionic strength and pH.

In addition, in order to improve half-life as well as membrane penetration, a large number of variations in polynucleotide backbones  
20 have been done, nevertheless with little success.

Oligonucleotides can be modified either in the base, the sugar or the phosphate moiety. These modifications include, for example, the use of methylphosphonates, monothiophosphates, dithiophosphates, phosphoramidates, phosphate esters, bridged phosphorothioates, bridged  
25 phosphoramidates, bridged methylenephosphonates, dephospho internucleotide analogs with siloxane bridges, carbonate bridges, carboxymethyl ester bridges, carbonate bridges, carboxymethyl ester bridges, acetamide bridges, carbamate bridges, thioether bridges, sulfoxy bridges, sulfono bridges, various "plastic" DNAs,  $\alpha$ -anomeric bridges and  
30 borane derivatives.

International patent application WO 89/12060 discloses various building blocks for synthesizing oligonucleotide analogs, as well as oligonucleotide analogs formed by joining such building blocks in a defined sequence. The building blocks may be either "rigid" (i.e.,  
35 containing a ring structure) or "flexible" (i.e., lacking a ring structure). In both cases, the building blocks contain a hydroxy group and a mercapto group, through which the building blocks are said to join to form oligonucleotide analogs. The linking moiety in the oligonucleotide

analogs is selected from the group consisting of sulfide (-S-), sulfoxide (-SO-), and sulfone (-SO<sub>2</sub>-).

International patent application WO 92/20702 describe an acyclic oligonucleotide which includes a peptide backbone on which any selected chemical nucleobases or analogs are stringed and serve as coding characters as they do in natural DNA or RNA. These new compounds, known as peptide nucleic acids (PNAs), are not only more stable in cells than their natural counterparts, but also bind natural DNA and RNA 50 to 100 times more tightly than the natural nucleic acids cling to each other. PNA oligomers can be synthesized from the four protected monomers containing thymine, cytosine, adenine and guanine by Merrifield solid-phase peptide synthesis. In order to increase solubility in water and to prevent aggregation, a lysine amide group is placed at the C-terminal region and may be pegylated.

Thus, antisense technology requires pairing of messenger RNA with an oligonucleotide to form a double helix that inhibits translation. The concept of antisense-mediated gene therapy was already introduced in 1978 for cancer therapy. This approach was based on certain genes that are crucial in cell division and growth of cancer cells. Synthetic fragments of genetic substance DNA can achieve this goal. Such molecules bind to the targeted gene molecules in RNA of tumor cells, thereby inhibiting the translation of the genes and resulting in dysfunctional growth of these cells. Other mechanisms has also been proposed. These strategies have been used, with some success in treatment of cancers, as well as other illnesses, including viral and other infectious diseases. Antisense oligonucleotides are typically synthesized in lengths of 13-30 nucleotides. The life span of oligonucleotide molecules in blood is rather short. Thus, they have to be chemically modified to prevent destruction by ubiquitous nucleases present in the body. Phosphorothioates are very widely used modification in antisense oligonucleotide ongoing clinical trials. A new generation of antisense molecules consist of hybrid antisense oligonucleotide with a central portion of synthetic DNA while four bases on each end have been modified with 2'O-methyl ribose to resemble RNA. In preclinical studies in laboratory animals, such compounds have demonstrated greater stability to metabolism in body tissues and an improved safety profile when compared with the first-generation unmodified phosphorothioate. Doses of other nucleotide analogs have also been tested in antisense technology.

RNA oligonucleotides may also be used for antisense inhibition as they form a stable RNA-RNA duplex with the target, suggesting efficient inhibition. However, due to their low stability RNA oligonucleotides are typically expressed inside the cells using vectors designed for this purpose.

5 This approach is favored when attempting to target a mRNA that encodes an abundant and long-lived protein.

Recent scientific publications have validated the efficacy of antisense compounds in animal models of hepatitis, cancers, coronary artery restenosis and other diseases. The first antisense drug was recently  
10 approved by the FDA. This drug Fomivirsen, developed by Isis, is indicated for local treatment of cytomegalovirus in patients with AIDS who are intolerant of or have a contraindication to other treatments for CMV retinitis or who were insufficiently responsive to previous treatments for CMV retinitis (Pharmacotherapy News Network).

15 Several antisense compounds are now in clinical trials in the United States. These include locally administered antivirals, systemic cancer therapeutics. Antisense therapeutics has the potential to treat many life-threatening diseases with a number of advantages over traditional drugs. Traditional drugs intervene after a disease-causing protein is formed.  
20 Antisense therapeutics, however, block mRNA transcription/translation and intervene before a protein is formed, and since antisense therapeutics target only one specific mRNA, they should be more effective with fewer side effects than current protein-inhibiting therapy.

A second option for disrupting gene expression at the level of  
25 transcription uses synthetic oligonucleotides capable of hybridizing with double stranded DNA. A triple helix is formed. Such oligonucleotides may prevent binding of transcription factors to the gene's promoter and therefore inhibit transcription. Alternatively, they may prevent duplex unwinding and, therefore, transcription of genes within the triple helical  
30 structure.

Thus, according to a further aspect of the present invention there is provided a pharmaceutical composition comprising the antisense oligonucleotide described herein and a pharmaceutically acceptable carrier. The pharmaceutically acceptable carrier can be, for example, a  
35 liposome loaded with the antisense oligonucleotide. Formulations for topical administration may include, but are not limited to, lotions, ointments, gels, creams, suppositories, drops, liquids, sprays and powders. Conventional pharmaceutical carriers, aqueous, powder or oily bases,

thickeners and the like may be necessary or desirable. Compositions for oral administration include powders or granules, suspensions or solutions in water or non-aqueous media, sachets, capsules or tablets. Thickeners, diluents, flavorings, dispersing aids, emulsifiers or binders may be desirable. Formulations for parenteral administration may include, but are not limited to, sterile aqueous solutions which may also contain buffers, diluents and other suitable additives.

According to still a further aspect of the present invention there is provided a ribozyme comprising the antisense oligonucleotide described herein and a ribozyme sequence fused thereto. Such a ribozyme is readily synthesizable using solid phase oligonucleotide synthesis.

Ribozymes are being increasingly used for the sequence-specific inhibition of gene expression by the cleavage of mRNAs encoding proteins of interest. The possibility of designing ribozymes to cleave any specific target RNA has rendered them valuable tools in both basic research and therapeutic applications. In the therapeutics area, ribozymes have been exploited to target viral RNAs in infectious diseases, dominant oncogenes in cancers and specific somatic mutations in genetic disorders. Most notably, several ribozyme gene therapy protocols for HIV patients are already in Phase 1 trials. More recently, ribozymes have been used for transgenic animal research, gene target validation and pathway elucidation. Several ribozymes are in various stages of clinical trials. ANGIOZYME was the first chemically synthesized ribozyme to be studied in human clinical trials. ANGIOZYME specifically inhibits formation of the VEGF-r (Vascular Endothelial Growth Factor receptor), a key component in the angiogenesis pathway. Ribozyme Pharmaceuticals, Inc., as well as other firms have demonstrated the importance of anti-angiogenesis therapeutics in animal models. HEPTAZYME, a ribozyme designed to selectively destroy Hepatitis C Virus (HCV) RNA, was found effective in decreasing Hepatitis C viral RNA in cell culture assays (Ribozyme Pharmaceuticals, Incorporated - WEB home page).

According to still another aspect of the present invention there is provided an antibody comprising an immunoglobulin specifically recognizing and binding a polypeptide at least 60 %, preferably at least 65 %, more preferably at least 70 %, still preferably at least 75 %, yet preferably at least 80 %, more preferably at least 85 %, more preferably at least 90 %, most preferably at least 95 % - 100 % homologous (identical + similar) to SEQ ID NOs:3, 5, 7 or portions thereof using as determined

using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3). According to a preferred embodiment of this aspect of the present invention the antibody specifically recognizing and binding the polypeptides set forth in SEQ ID NOs:3, 5, 7 or portions thereof.

The present invention can utilize serum immunoglobulins, polyclonal antibodies or fragments thereof, (i.e., immunoreactive derivative of an antibody), or monoclonal antibodies or fragments thereof. Monoclonal antibodies or purified fragments of the monoclonal antibodies having at least a portion of an antigen binding region, including such as Fv, F(abl)2, Fab fragments (Harlow and Lane, 1988 Antibody, Cold Spring Harbor), single chain antibodies (U.S. Patent 4,946,778), chimeric or humanized antibodies and complementarily determining regions (CDR) may be prepared by conventional procedures. Purification of these serum immunoglobulins antibodies or fragments can be accomplished by a variety of methods known to those of skill including, precipitation by ammonium sulfate or sodium sulfate followed by dialysis against saline, ion exchange chromatography, affinity or immunoaffinity chromatography as well as gel filtration, zone electrophoresis, etc. (see Goding in, Monoclonal Antibodies: Principles and Practice, 2nd ed., pp. 104-126, 1986, Orlando, Fla., Academic Press). Under normal physiological conditions antibodies are found in plasma and other body fluids and in the membrane of certain cells and are produced by lymphocytes of the type denoted B cells or their functional equivalent. Antibodies of the IgG class are made up of four polypeptide chains linked together by disulfide bonds. The four chains of intact IgG molecules are two identical heavy chains referred to as H-chains and two identical light chains referred to as L-chains. Additional classes includes IgD, IgE, IgA, IgM and related proteins.

Methods for the generation and selection of monoclonal antibodies are well known in the art, as summarized for example in reviews such as Tramontano and Schloeder, Methods in Enzymology 178, 551-568, 1989. A recombinant protein of the present invention may be used to generate antibodies *in vitro*. More preferably, the recombinant protein of the present invention is used to elicit antibodies *in vivo*. In general, a suitable host animal is immunized with the recombinant protein of the present invention. Advantageously, the animal host used is a mouse of an inbred

strain. Animals are typically immunized with a mixture comprising a solution of the recombinant protein of the present invention in a physiologically acceptable vehicle, and any suitable adjuvant, which achieves an enhanced immune response to the immunogen. By way of example, the primary immunization conveniently may be accomplished with a mixture of a solution of the recombinant protein of the present invention and Freund's complete adjuvant, said mixture being prepared in the form of a water in oil emulsion. Typically the immunization may be administered to the animals intramuscularly, intradermally, subcutaneously, intraperitoneally, into the footpads, or by any appropriate route of administration. The immunization schedule of the immunogen may be adapted as required, but customarily involves several subsequent or secondary immunizations using a milder adjuvant such as Freund's incomplete adjuvant. Antibody titers and specificity of binding to the recombinant protein can be determined during the immunization schedule by any convenient method including by way of example radioimmunoassay, or enzyme linked immunosorbant assay, which is known as the ELISA assay. When suitable antibody titers are achieved, antibody producing lymphocytes from the immunized animals are obtained, and these are cultured, selected and cloned, as is known in the art. Typically, lymphocytes may be obtained in large numbers from the spleens of immunized animals, but they may also be retrieved from the circulation, the lymph nodes or other lymphoid organs. Lymphocytes are then fused with any suitable myeloma cell line, to yield hybridomas, as is well known in the art. Alternatively, lymphocytes may also be stimulated to grow in culture, and may be immortalized by methods known in the art including the exposure of these lymphocytes to a virus, a chemical or a nucleic acid such as an oncogene, according to established protocols. After fusion, the hybridomas are cultured under suitable culture conditions, for example in multiwell plates, and the culture supernatants are screened to identify cultures containing antibodies that recognize the hapten of choice. Hybridomas that secrete antibodies that recognize the recombinant protein of the present invention are cloned by limiting dilution and expanded, under appropriate culture conditions. Monoclonal antibodies are purified and characterized in terms of immunoglobulin type and binding affinity.

Additional objects, advantages, and novel features of the present invention will become apparent to one ordinarily skilled in the art upon examination of the following examples, which are not intended to be limiting. Additionally, each of the various embodiments and aspects of the present invention as delineated hereinabove and as claimed in the claims section below finds experimental support in the following examples.

### EXAMPLES

Reference is now made to the following examples, which together with the above descriptions, illustrate the invention in a non limiting fashion.

Generally, the nomenclature used herein and the laboratory procedures in recombinant DNA technology described below are those well known and commonly employed in the art. Standard techniques are used for cloning, DNA and RNA isolation, amplification and purification. Generally enzymatic reactions involving DNA ligase, DNA polymerase, restriction endonucleases and the like are performed according to the manufacturers' specifications. These techniques and various other techniques are generally performed according to Sambrook et al., molecular Cloning - A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y. (1989), which is incorporated herein by reference. Other general references are provided throughout this document. The procedures therein are believed to be well known in the art and are provided for the convenience of the reader. All the information contained therein is incorporated herein by reference.

#### *Materials and Experimental Methods*

The following protocols and experimental details are referenced in the Examples that follow:

#### *Primers list:*

hn11116	5'-GGAGAGCAAGTCTGTGTTGATTC-3'	(SEQ ID NO:10)
hn11230	5'-CACTGGTAGCCATGAGTGTGAG-3'	(SEQ ID NO:11)
hn1u350	5'-TTGGTCATCCCTCCAGTCACCA-3'	(SEQ ID NO:12)
pn9-312u	5'-CTTGCCTGTAGACAGAGCTGCAG-3'	(SEQ ID NO:14)
hpu-685	5'-GAGCAGCCAGGTGAGCCCAAGA-3'	(SEQ ID NO:16)
hpl967	5'-TCAGATGCAAGCAGCAACTTTGGC-3'	(SEQ ID NO:17)
mn1u118	5'-CACCTGATGTCATGCTGGAG-3'	(SEQ ID NO:18)
mn11563	5'-CATCTAGGAGAGCAATGACGTTC-3'	(SEQ ID NO:19)

Ap1 5'-CCATCCTAATACGACTCACTATAGGGC-3' (SEQ ID NO:20)

Ap2 5'-ACTCACTATAGGGCTCGAGCGGC-3' (SEQ ID NO:21)

***Southern analysis:***

Genomic DNA was extracted from animal or from human blood  
5 using Blood and cell culture DNA maxi kit (Qiagene). DNA was digested  
with *Eco*RI, separated by gel electrophoresis and transferred to a nylon  
membrane Hybond N+ (Amersham). PCR products underwent a similar  
procedure. Hybridization was performed at 68° C in 6 x SSC, 1 % SDS, 5  
x Denharts, 10 % dextran sulfate, 100 µg/ml salmon sperm DNA, and <sup>32</sup>p  
10 labeled probe. Pn9, a 1.7 kb fragment, which contain the entire open  
reading frame except for a deletion of 162 nucleotides (del:473-634, SEQ  
ID NO:1) was used as a probe. Following hybridization, the membrane  
was washed with 3 x SSC, 0.1 % SDS, at 68 °C and exposed to X-ray film  
for 3 days. Membranes were then washed with 0.1 x SSC, 0.1 % SDS, at  
15 68 °C and were re-exposed for 4 days.

***RT-PCR:***

RNA was prepared using TRI-Reagent (Molecular research center  
Inc.) according to the manufacturer instructions. 1.25 µg were taken for  
reverse transcription reaction using SuperScriptII Reverse transcriptase  
20 (Gibco BRL) and Oligo (dT)<sub>15</sub> primer (SEQ ID NO:22), (Promega).  
Amplification of the resultant first strand cDNA was performed with *Taq*  
polymerase (Promega) or with Expand high fidelity (Boehringer  
Mannheim).

***cDNA Sequence analysis:***

Sequence determinations were performed with vector specific and  
gene specific primers, using an automated DNA sequencer (Applied  
Biosystems, model 373A). Each nucleotide was read from at least two  
independent primers. Computation and sequence analysis and alignments  
were done using the DNA sequence analysis software package developed  
30 by the Genetic Computer Group (GCG) at the university of Wisconsin.  
Alignments of two sequences were performed using Bestfit (gap creation  
penalty - 12, gap extension penalty - 4) or with Gap program (gap creation  
penalty - 50, gap extension penalty - 3).

***Tissue distribution:***

35 Tissue distribution of the *hnhp1* transcript was determined by semi-  
quantitative PCR. cDNA panels were obtained from Clontech. PCR was  
performed with the gene specific primers hn1u350 (SEQ ID NO:12) and  
hn1l116 (SEQ ID NO:10). PCR program was as follows: 94 °C, 3



minutes, followed by 40 cycles of 94 °C, 45 seconds, 64 °C, 1 minute, 72 °C, 1 minute. Samples were taken for further analysis following 25, 30, 35 and 40 cycles.

***Chromosome localization:***

5 Chromosome localization of *hnhp1* was performed using the radiation hybrid panel Stanford G3. This panel was provided by the human genome center at the Weizmann Institute. A 225 bp genomic fragment of *hnhp1* gene was amplified using the gene specific primers *hn1u350* (SEQ ID NO:12) and *hn11116* (SEQ ID NO:10). PCR program  
10 was as follows: 94 °C, 3 minutes, followed by 39 cycles of 94 °C 45 seconds, 64 °C, 1 minute, 72 °C, 1 min. Analysis of results was done through the RH server at the Stanford human genome center.

**EXAMPLE 1**

15 ***Cloning an EST for a novel heparanase gene***

The entire amino acid sequence of human heparanase (SEQ ID NO:9) was used to screen human EST database for homologous sequences. Screening was performed using the BLAST 2.0 server at the NCBI, basic BLAST search, *tblastn* program.

20 A distantly homologous fragment was pooled out, accession number AI222323, IMAGE clone number 1843155 from Soares\_NFL\_T\_GBC\_S1 Homo Sapiens cDNA library prepared from testis B-cells and fetal lungs. The search values for this sequence were as follows: Score = 38.3 bits (87), Expect = 0.15 Identities = 16/36 (44 %),  
25 Positives = 22/36 (60 %). The sequence of accession number AI222323 contains 378 nucleotides of the 3' of clone 1843155 (complementary to nucleotides 165-543 of SEQ ID NO:23).

This clone was purchased from the IMAGE consortium. It contained an insert of 560 bp (SEQ ID NO:23). The entire nucleotide  
30 sequence was determined and compared to the *hpa* cDNA encoding human heparanase. The homology between clone 1843155 and *hpa* cDNA was restricted to the 3' region of the cDNA clone. There was 59 % homology between nucleotides 99-275 of clone 1843155 (SEQ ID NO:23), and 1532-1708 of *hpa* (SEQ ID NO:24). The deduced amino acid  
35 sequence of this region had 60 % homology (identical + similar) to amino acids 488-542 (SEQ ID NO:9) of human heparanase. The downstream sequence (nucleotides 276-560, SEQ ID NO:23) represents a 3' untranslated region and a poly A tail. The upstream sequence, nucleotides

1-98 (SEQ ID NO:23) was unrelated to heparanase. This unrelated sequence was found to be identical to a different cDNA clone from the same library. Therefore, the human EST clone 1843155, obtained from the IMAGE consortium is assumed to be a chimera, which contains two  
5 unrelated partial cDNAs ligated to a single vector.

## EXAMPLE 2

### *Cloning a cDNA for a novel heparanase gene*

In order to isolate the entire cDNA, three primers were designed  
10 according to the sequence of clone 1843155. The cDNA was amplified from placenta cDNA by Marathon RACE (rapid amplification of cDNA ends) (Clontech, Palo Alto, California) according to the manufacturer instructions. The first cycle was performed with the gene specific primer hn11116 (SEQ ID NO:10) and the universal primer Ap1 (SEQ ID NO:20).  
15 The second cycle was performed with the gene specific primer hn11230 (SEQ ID NO:11) and the universal primer Ap2 (SEQ ID NO:21). Following amplification, a difused band of approximately 1.7 kb was obtained. This cDNA amplification product was subcloned into pGEM T-easy (Promega, Madison, WI) and the nucleotide sequences of three  
20 independent clones pn5, pn6 and pn9 were determined. The consensus sequence of the longest cDNA, pn6, appears in Figure 1 (SEQ ID NOs:1, 2 and 3). It is 2060 nucleotide long and it contains an open reading frame of 1776 nucleotides, which encodes a polypeptide of 592 amino acids, with a calculated molecular weight of 66.5 kDa. The newly cloned gene was  
25 designated *hnhp1*. The two shorter forms, pn9 and pn5 and their deduced amino acid sequences are set forth in SEQ ID NOs:4 and 6 and SEQ ID NO:5 and 7, respectively. Pn9 and pn5 were identical to pn6, however each one of them contained an in frame deletion as a result of alternative splicing. Pn9 contains a deletion of 162 nucleotides, 473-634 of SEQ ID  
30 NO:1, which correspond to amino acids 150-203 of SEQ ID NO:3. As a result pn9 encodes a putative polypeptide of 538 amino acids (SEQ ID NO:5) having a calculated molecular weight of 60.4 kDa. Pn5 contains a deletion of 336 nucleotides, 473-808 of SEQ ID NO:1, which correspond to amino acids 150-261 of SEQ ID NO:3, thus, it encodes a putative  
35 polypeptides of 480 amino acids (SEQ ID NO:7) having a calculated molecular weight of 53.9 kDa. The 11<sup>th</sup> amino acid residue of SEQ ID NO:3 is methionine. It is generally accepted that the first methionine serves as a translation start site in mammals, however, the nucleotides

surrounding the second ATG fit better with the Kozak consensus sequence for translation start site. Translation may thus start at the second methionine and produce a protein of 581 amino acids with calculated molecular weight of 65.4 kDa. The presence of transcripts of variable length was confirmed by PCR amplification of the *hn1hp* cDNA using two gene specific primers: pn9-312u (SEQ ID NO:14) which is located close to the 5' end and hn11230 (SEQ ID NO:11) which overlaps the stop codon at the 3' end of the open reading frame. Amplification was performed from Marathon ready cDNA prepared from placenta and from testis. The PCR products are shown in figure 3. Four bands were obtained from placenta: two major bands of 1.45 and 1.6 kb, similar to pn9 and pn6 and two minor bands, one of 1.35 kb, similar to pn5 and a second one of 1.8 kb. The sequence of the latter has not yet been determined. Amplification of testis cDNA resulted in a different pattern. Four bands of 1.35, 1.65, 1.85 and 2.05 kb were observed and a minor one of 1.5 kb. The various forms appear to represent products of alternative splicing. Since the deletions characterized so far retain an open reading frame, the translation products of the various cDNAs may constitute a protein family. The comparison between the amino acid sequence of *hnhp1* and heparanase is shown in Figure 3. Using the gap program of the GCG package which aligns the entire amino acid sequences, the homology between the two proteins is 45.5 % identity and 7.3 % similarity, total homology of 52.8 % (gap creation penalty - 50, gap extension penalty - 3). The BestFit program defines the region of the best homology between the two sequences. Using this program, the homology between the two amino acid sequences starts at position 63 of *hn1hp1* (SEQ ID NO:3) and position 41 of heparanase (SEQ ID NO:9) and is 47.5 % identity and 7.8 % similarity, i.e. homology of 55.3 %. The homology between the nucleotide sequences of *hnhp1* and *hpa* is 57 % as calculated by the BestFit program. The homologous region is located between nucleotides 638-1812 of *hnhp1* (SEQ ID NO:1) and nucleotides 564-1708 of *hpa* (SEQ ID NO:24). Using the Gap program the homology is 51 % over the entire sequence gap creation penalty - 50, gap extension penalty - 3.

### EXAMPLE 3

#### *Zoo blot*

*Hnhp1* cDNA was used as a probe to detect homologous sequences in human DNA and in DNA of various animals. The autoradiogram of the

Southern analysis is presented in Figure 4. Several bands were detected in human DNA. Several intense bands were detected in all mammals, while faint bands were detected in chicken. This correlates with the phylogenetic relation between human and the tested animals. The intense bands indicate that *hnhp1* is conserved among mammals as well as in more genetically distant organisms. The multiple bands patterns suggest that in all animals, *hnhp1* locus occupies a large genomic region. Several specific bands disappeared after stringent wash. These may represent homologous sequences and suggest the existence of a gene family, which can be isolated based on their homology to the human *hnhp1* reported here.

#### EXAMPLE 4

##### *comparison to heparanase via cross hybridization*

In order to check the capability of *hpa* and *hnhp1* to cross hybridize under low stringency conditions, the entire coding region of the human *hpa* and *hnhp1* were amplified by PCR. Human *hpa* was amplified from platelets mRNA by RT-PCR using the primers hpu-685 (SEQ ID NO:16) and hpl967 (SEQ ID NO:17), and *hnhp1* was amplified from testis using the primers hn11230 (SEQ ID NO:11) and pn9-312u (SEQ ID NO:14). The products were quantified and samples of 100 pg and 1 ng were run on agarose gel and subjected to Southern hybridization. The membranes were probed with <sup>32</sup>p labeled *hpa* cDNA and with *hnhp1* cDNA. No cross hybridization was observed (Figure 5) even after over exposure for 5 days. Since *hpa* is the most similar sequence known today to that of *hnhp1*, this experiment indicates that the bands detected in the autoradiograph of Figure 4 are of the *hnhp1* gene or of yet unknown sequences homologous thereto, which might constitute a gene family. This further indicated that such sequences are isolatable using the *hnhp1* as a probe to screen the relevant libraries, or using *hnhp1* derived PCR primers to amplify the relevant cDNA or DNA sequences.

#### EXAMPLE 5

##### *Chromosome localization*

The chromosome localization of *hnhp1* was determined using G3 radiation hybrid panel. *Hnhp1* was amplified from 83 human/mouse radiation hybrids. The results were analyzed by the RH server and the *hnhp1* gene was mapped to chromosome 10, next to the marker SHGC-

57721. The results also indicated a possibility of a second copy of the gene.

### EXAMPLE 6

#### *Expression Pattern of hnhp1*

The tissue distribution of *hnhp1* transcripts was determined using calibrated human cDNA panels (Clontech, Palo Alto, Ca). The results are shown in Table 1 below. Expression level is generally low. PCR products were clearly observed only after 40 cycles of amplification.

TABLE 1

<u>Tissue</u>	<u>hn1 (40 cycles)</u>
Bone marrow	
Liver	
Lymph node	+
Leukocytes	
Spleen	+
Thymus	
Tonsil	
Colon	+
Ovary	+
Prostate	++
Small intestine	++
Testis	+++

### EXAMPLE 7

#### *cloning of a Mouse homologue*

Screening of the mouse EST database with the amino acid sequence of heparanase as well as of *hnhp1* pooled out a mouse EST clone, which shares distant homology with heparanase and a remarkably high homology with *hnhp1*. The EST clone 1378452 accession number AI019269 from mouse thymus was 351 nucleotide long and it is set forth in SEQ ID NO:8. It has 61-63 % identity over 161 nucleotides (191-351, SEQ ID NO:8) to the human (SEQ ID NO:24) and mouse (SEQ ID NO:15) *hpa* nucleotide sequences, and 93 % to *hnhp1* nucleotide sequence (SEQ ID NO:1) using the BestFit program of the GCG package. The nucleotide sequence of this clone did not contain an open reading frame. Two frame shifts were identified in the sequence found in the EST database, as compared to the

*hnhp1* sequence. This frame shifts were later confirmed by nucleotide sequence analysis of this clone as well as by isolation of this fragment from BL6 mouse melanoma cells and determination of its nucleotide sequence. This mouse gene is transcribed at very low levels. Low levels of expression were indicated as no amplification products were obtained following 40 cycles of PCR from mouse cDNA panel (Clontech, Palo Alto, Ca) which included cDNA from mouse heart, brain, spleen, lung, liver, skeletal muscle, kidney, testis and embryos of 7, 11, 15, and 17 days. The amplification was performed using the gene specific primers mn1u118 (SEQ ID NO:18) and mn11563 (SEQ ID NO:19).

### EXAMPLE 8

#### *Expression of hnhp1 in mammalian cells*

A mammalian expression vector was constructed in order to over-express *hnhp1* in human cells. To enable detection of the Hnhp1 translation product, the *hnhp1* expression vector was designed to encode a C-terminal tagged hn1 protein. A DNA sequence, which encodes eight amino acids FLAG (Kodak), was fused to the 3' end of the *hnhp1* open reading frame.

Fusion of the FLAG sequence to the *hnhp1* coding sequence was generated by PCR amplification using the primer: hn1-c-flag: 5'-

A-3' (SEQ ID NO:25) and the primer: pn9-312u (SEQ ID NO:14). The PCR program was as follows: 94 °C, 3 min followed by 5 cycles of : 94 °C, 45 seconds, 50 °C, 45 seconds and 72 °C, 2 minutes, and then 32 cycles of 94 °C, 45 seconds, 64 °C, 45 seconds and 72 °C, 2 min.

The amplification product was subcloned into pGEM-T-easy, and the sequence was verified. The resulting plasmids were designated pGEM-pn6F and pGEM-pn9F.

Two constructs were generated in pSI mammalian expression vector (Promega): the first contained the complete *hnhp1* sequence (pn6) and the second contained the alternative splice form (pn9). The pSI-pn6 expression vector was constructed by triple ligation of the following fragments: an EcoRI – BamHI fragment, which contains the 5' end of hn1-pn6, excised from pGem-T-easy-pn9, a BamHI – NotI fragment which contains the 3' FLAG tagged *hnhp1*, excised from pGEM-pn6F and pSI digested with EcoRI – NotI.

The pSI-pn9 expression vector was constructed similarly, by triple ligation of the following fragments: an EcoRI – SspI fragment, which contains the 5' end of hnnp1-pn6, excised from pGem-T-easy-pn9, an SspI – NotI fragment, which contains the 3' FLAG tagged hnnp1, excised from pGem-pn6F and pSI digested with EcoR I – Not I.

The resulting plasmids were transfected into human embryonal kidney 293 cells, using the Fugene transfection reagent (Boehringer Mannheim). Forty-eight hours following transfection cells were harvested and proteins were analysed by western blot. Cell lysates of  $2.5 \times 10^5$  were separated by SDS-PAGE, transferred onto a nylon membrane and incubated with anti FLAG antibody 1:1000 dilution (Kodak anti FLAG M2 cat: IB13025, final concentration 10 µg/ml). Proteins of approximately 65 kDa and 60 kDa were detected in cells transfected with pSI-pn6F and pSI-pn9F respectively. These proteins are similar in size to those predicted by the calculated molecular weight for the translation products of corresponding open reading frames. It is demonstrated that both the entire hnnp1 cDNA and the pn9 splice form are successfully transcribed and translated in human 293 cells. However, unlike heparanase the Hnnp1 protein products do not undergo major processing in these cells.

Although the invention has been described in conjunction with specific embodiments thereof, it is evident that many alternatives, modifications and variations will be apparent to those skilled in the art. Accordingly, it is intended to embrace all such alternatives, modifications and variations that fall within the spirit and broad scope of the appended claims. All publications cited herein are incorporated by reference in their entirety.

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## WHAT IS CLAIMED IS:

1. An isolated nucleic acid comprising a polynucleotide hybridizable with SEQ ID NOs:1, 4, 6 or portions thereof at 68 °C in 6 x SSC, 1 % SDS, 5 x Denharts, 10 % dextran sulfate, 100 µg/ml salmon sperm DNA, and <sup>32</sup>p labeled probe and wash at 68 °C with 3 x SSC and 0.1 % SDS.

2. An isolated nucleic acid comprising a polynucleotide at least 60 % identical with SEQ ID NOs:1, 4, 6 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

3. The isolated nucleic acid of claim 2, wherein said polynucleotide is as set forth in SEQ ID NOs:1, 4, 6 or portions thereof.

4. An isolated nucleic acid comprising a polynucleotide encoding a polypeptide being at least 60 % homologous with SEQ ID NOs:3, 5, 7 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

5. A recombinant protein comprising a polypeptide encoded by the polynucleotide of claim 1.

6. A recombinant protein comprising a polypeptide encoded by the polynucleotide of claim 2.

7. A recombinant protein comprising a polypeptide encoded by the polynucleotide of claim 3.

8. A recombinant protein comprising a polypeptide encoded by the polynucleotide of claim 4.

9. A recombinant protein comprising a polypeptide at least 60 % homologous with SEQ ID NOs:3, 5, 7 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

10. The recombinant protein of claim 9, wherein said polypeptide is as set fourth in SEQ ID NOs:3, 5, 7 or portions thereof.

11. A nucleic acid construct comprising the isolated nucleic acid of claim 1.

12. A nucleic acid construct comprising the isolated nucleic acid of claim 2.

13. A nucleic acid construct comprising the isolated nucleic acid of claim 3.

14. A nucleic acid construct comprising the isolated nucleic acid of claim 4.

15. A host cell comprising the nucleic acid construct of claim 11.

16. A host cell comprising the nucleic acid construct of claim 12.

17. A host cell comprising the nucleic acid construct of claim 13.

18. A host cell comprising the nucleic acid construct of claim 14.

19. An antisense oligonucleotide comprising a polynucleotide or a polynucleotide analog of at least 10 bases being hybridizable *in vivo*, under physiological conditions, with:

- (i) a portion of a polynucleotide strand encoding a polypeptide at least 60 % homologous with SEQ ID NOs:3, 5, 7 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3); or
- (ii) a portion of a polynucleotide strand at least 60 % identical with SEQ ID NOs:1, 4, 6 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).

20. A ribozyme comprising the antisense oligonucleotide of claim 19 and a ribozyme sequence.

21. An antisense nucleic acid construct comprising a promoter sequence and a polynucleotide sequence directing the synthesis of an antisense RNA sequence of at least 10 bases being hybridizable *in vivo*, under physiological conditions, with:

- (i) a portion of a polynucleotide strand encoding a polypeptide at least 60 % homologous with SEQ ID NOs:3, 5, 7 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3); or
- (ii) a portion of a polynucleotide strand at least 60 % identical with SEQ ID NOs:1, 4, 6 or portions thereof as determined using the Bestfit procedure of the DNA sequence analysis software package developed by the Genetic Computer Group (GCG) at the university of Wisconsin (gap creation penalty - 50, gap extension penalty - 3).



CGCTTAATTCTAGAAGGGATTGA	25
ATGAGGGTGCTTTGTGCCTTCCCTGAAGCCATGCCCTCCAGCAACTCCCGCCCCCGCG M R V L C A F P E A M P S S N S R P P A	85
TGCCTAGCCCCGGGGCTCTCTACTTGGCTCTGTTGCTCCATCTCTCCCTTTCCTCCAG C L A P G A L Y L A L L L H L S L S S Q	145
GCTGGAGACAGGAGACCCTTGCTGTAGACAGAGCTGCAGGTTTGAAGGAAAAGACCCTG A G D R R P L P V D R A A G L K E K T L	205
ATTCTACTTGATGTGAGACCAAGAACCAGTCAGGACAGTCAATGAGAATTCTCTCTCT I L L D V S T K N P V R T V N E N F L S	265
CTGCAGCTGGATCCGTCCATCATTGATGATGGCTGGCTCGATTTCTAAGCTCCAAGCGC L Q L D P S I I H D G W L D F L S S K R	325
TTGGTGACCCCTGGCCCGGGGACTTTCGCCCGCCTTCTGCGCTTCGGGGGCAAAAGGACC L V T L A R G L S P A F L R F G G K R T	385
GACTTCCTGCAGTCCAGAACCTGAGGAACCCGGCGAAAAGCCGGGGGCCGGGCCCG D F L Q F Q N L R N P A K S R G G P G P	445
GATTACTACTCTCAAAACTATGAGGATGACATTGTTTGAAGTATGTTGCCTTAGATAAA D Y Y L K N Y E D D I V R S D V A L D K	505
CAGAAAGGCTGCAAGATTGCCAGCACCCCTGATGTTATGCTGGAGCTCCAAGGGAGAAG Q K G C K I A Q H P D V M L E L Q R E K	565
GCAGCTCAGATGCATCTGGTTCTTCTAAAGGAGCAATTCTCCAATACTTACAGTAATCTC A A Q M H L V L L K E Q F S N T Y S N L	625
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CTGATATTGCTCTAAATGCACTGCGTCGTAATCCCAATAACTCCTGGAACAGTTCTAGT L I F A L N A L R R N P N N S W N S S S	745
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AATGAGCCAAATAACTATCGGACCATGCATGGCCGGGCAGTAAATGGCAGCCAGTTGGGA N E P N N Y R T M H G R A V N G S Q L G	865
AAGGATTACATCCAGCTGAAGAGCCTGTTGCAGCCCATCCGGATTATTCAGAGCCAGC K D Y I Q L K S L L Q P I R I Y S R A S	925
TTATATGGCCCTAATATTGGCGCGCGAGGAAGAATGTCATCGCCCTCTAGATGGATTC L Y G P N I G R P R K N V I A L L D G F	985
ATGAAGGTGGCAGGAAGTACAGTAGATGCAGTTACCTGGCAACATTGCTACATTGATGGC M K V A G S T V D A V T W Q H C Y I D G	1045
CGGGTGGTCAAGTGATGGACTTCCTGAAAACCTCGCCTGTTAGACACACTCTCTGACCAG R V V K V M D F L K T R L L D T L S D Q	1105
ATTAGGAAAATTCAGAAAGTGGTTAATACATACACTCCAGGAAAGAAGATTGGCTTGAA I R K I Q K V V N T Y T P G K I W L E	1165
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TTCTTATGGTTGAACACTTTAGGAATGCTGGCCAATCAGGGCATTGATGTCGTGATACGG F L W L N T L G M L A N Q G I D V V I R	1285
CACTCATTTTTTGACCATGGATACAATCACCTCGTGGACCAGAATTTTAACCCATTACCA H S F F D H G Y N H L V D Q N F N P L P	1345
GACTACTGGCTCTCTCTCTCTACAAGCGCCTGATCGGCCCAAAGTCTTGCTGTGCAT D Y W L S L L Y K R L I G P K V L A V H	1405
GTGGCTGGGTCCAGCGGAAGCCACGGCCTGGCCGAGTGATCCGGGACAAACTAAGGATT V A G L Q R K P R P G R V I R D K L R I	1465

ATCATCAACTTGCATCGATCAAGAAAGAAAATCAAGCTGGCTGGGACTCTCAGAGACAAG	1585
I I N L H R S R K K I K L A G T L R D K	
CTGGTTCACCAAGTACCTGCTGCAGCCCTATGGGCAGGAGGGCCTAAAGTCCAAGTCAGTG	1645
L V H Q Y L L Q P Y G Q E G L K S K S V	
CAACTGAATGGCCAGCCCTTAGTGATGGTGGACGACGGGACCCTCCCAGAATTGAAGCCC	1705
Q L N G Q P L V M V D D G T L P E L K P	
CGCCCCCTTCGGGCCGGCCGGACATTGGTCATCCCTCCAGTCACCATGGGCTTTTTGTG	1765
R P L R A G R T L V I P P V T M G F F V	
GTCAAGAATGTCAATGCTTTGGCCTGCCGCTACCGATAAGCTATCCTCACACTCATGGCT	1825
V K N V N A L A C R Y R *	
ACCAAGTGGGCCTGCTGGGCTGCTTCCACTCCTCCACTCCAGTAGTATCCTCTGTTTTTCAG	1885
ACATCCTAGCAACCAGCCCTGCTGCCCCATCCTGCTGGAATCAACACAGACTTGCTCTC	1945
CAAAGAGACTAAATGTCATAGCGTGATCTTAGCCTAGGTAGGCCACATCCATCCCAAAGG	2005
AAAATGTAGACATCACCTGTACCTATATAAGGATAAAGGCATGTGTATAGAGCAA	2060

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1 MRVLCAPPEAMPSSNSRPPACLAPGALYLALLLHLSLSSQAGDRREL PVD 50
      | | | | | | | |
1 .....MLLRSKPALPPPLMLLLLGLGPLSPGALP 30
      | | | | | | | |
51 RAAGLKEKTLILLDVSTKNPVRTVNENFLSLQLDPSIIHD.GWLDLSSK 99
      | | . . .: || |. |. |. .|||. :| .: | .| | |
31 RPA..QAQDVVDLDFFTQEPLHLVSPSFLSVTIDANLATDPRFLILLGSP 78
      | | | | | | | |
100 RLVTIARGLSPAFLRFGGKRTDFLQFQNLRNPAKSRGGPGPDYLLKNYED 149
      :| | | | | | | | :| | | | | | | | | | | | | | |
79 KLRTIARGLSPAYLRFGGKTDFLIF....DPKKESTFEERSYQSQVNQ 124
      | | | | | | | |
150 DIVRSDVALDKQKGCKIAQHPDVMLELQREKAAQMHLVLLKEQFSNTYSN 199
      || | | | | | | | | | | | | | | | | | | | |
125 DI.....CKYGSIPPDVEEKLRLWPYQEQLLLREHYQKKFKN 162
      | | | | | | | |
200 LILTARSLDKLYNFADCSGLHLIFALNALRRNPNNSSWSSALSLLKYSA 249
      . |. | | |. | | | | | | | | | | | | | | | |
163 STYSRSSVDVLYTFANCSGLDLIFGLNALLRTADLQWNSSNAQLLLDYCS 212
      | | | | | | | |
250 SKKYNISWELGNEPNYRTMHGRAVNGSQLGKDYIQLKSLLOPIRIYSRA 299
      || | | | | | | | | | | | | | | | | | | | |
213 SKGYNISWELGNEPNSFLKKADIFINGSQLGEDFIQLHKLLRK.STFKNA 261
      | | | | | | | |
300 SLYGNIGRPRKNVIALLDGFMKVAGSTVDAVTWQHCYIDGRVVKVMDFL 349
      | | | | :|. | | : | | | | | | | | | | | |
262 KLYGPDVGQPRRKTAQMLKSFLKAGGEVIDSVTWHHYLLNGRTATREDFL 311
      | | | | | | | |
350 KTRLLDTLSQIRKIQKVNTYTPGKKIWLEGVVTTSAGGTNNLSDSYAA 399
      .| | | | | | | | | | | | | | | | | | | |
312 NPDVLDIFISSVQKVQVVESTRPQKQVWLGETSSAYGGGAPLLSDTFAA 361
      | | | | | | | |
400 GFLWLNTLGMLANQGIDVVIRHSFFDHGYNHLVDQNFNPLPDYWLSSLYK 449
      ||: ||. ||: | ||: ||. | | | | | | | | | | | |
362 GFMWLDKLGLSARMGIEVVMRQVFFGAGNYHLVDENFDPLPDYWLSSLEK 411
      | | | | | | | |
450 RLIGPKVLAVHVAGLQKRPGRVIRDKLRIYAHCTNHHNNHNYVRGSITL 499
      :|: | | | | | | | | | | | | | | | | | | |
412 KLVGTKVLMASVQGSKRR.....KLRVYLHCTNTDNPRYKEGDLTL 452
      | | | | | | | |
500 FIINLHRSRKKIKLAGTLRDKLVHQYLLQPYGQEGLSKSVQLNGQPLVM 549
      : | | | | | | | | | | | | | | | | | | | |
453 YAINLHNVTKYLRLPYPFSNKQVDKYLLRPLGPHGLLSKSVQLNGITLKM 502
      | | | | | | | |
550 VDDGTLPCLKPRPLRAGRTLVIPTMTGFFVVKVNVNALACRYR 592
      || | | | | | | | | | | | | | | | |
503 VDDQTLFPLMEKPLRPGSSGLPAFSYSFFVIRNAKVAACI. 543

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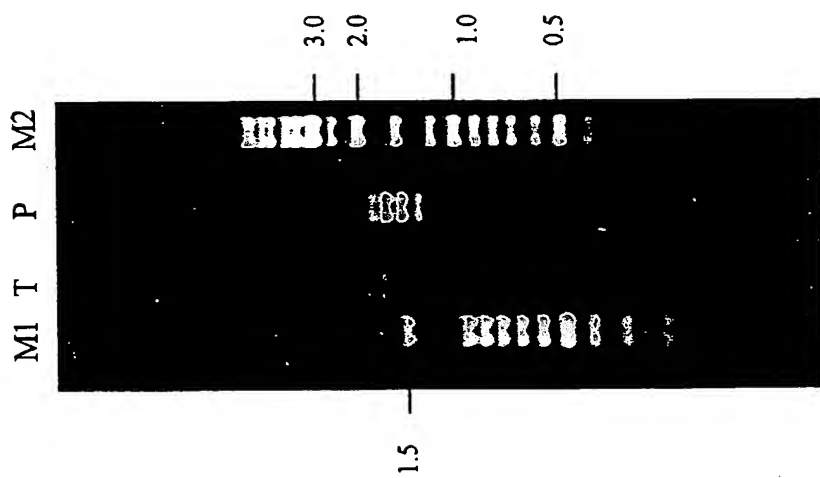


Figure 3

5/8

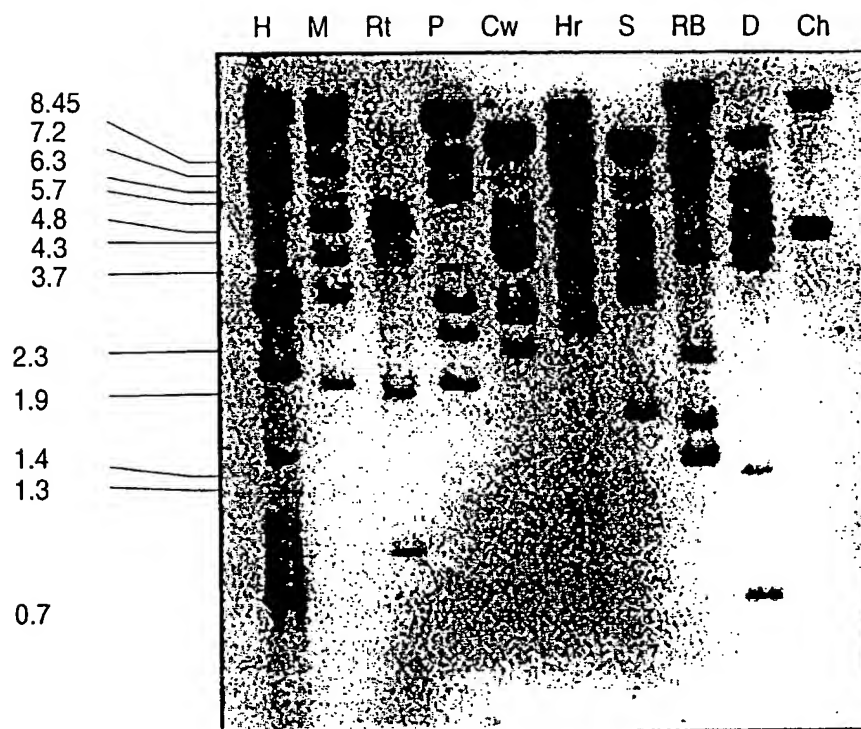
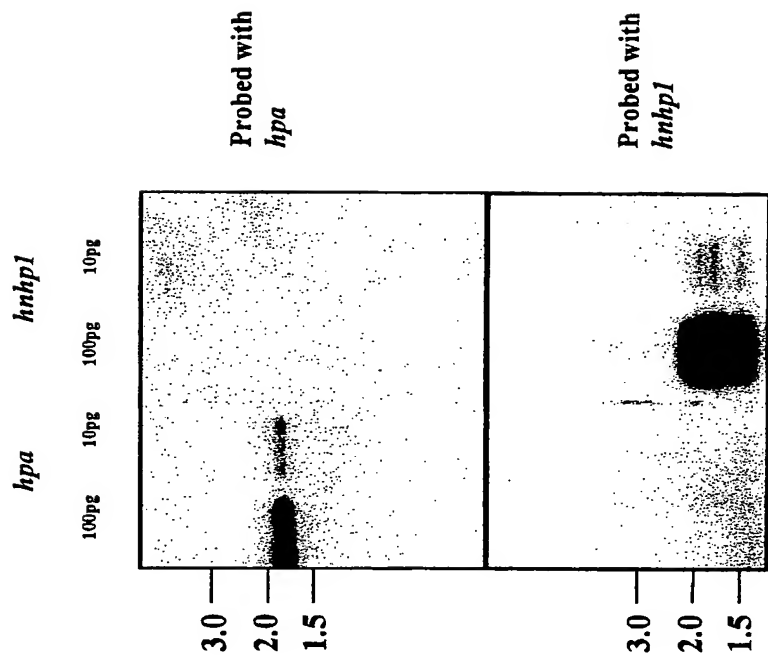


FIG.4

Figure 5



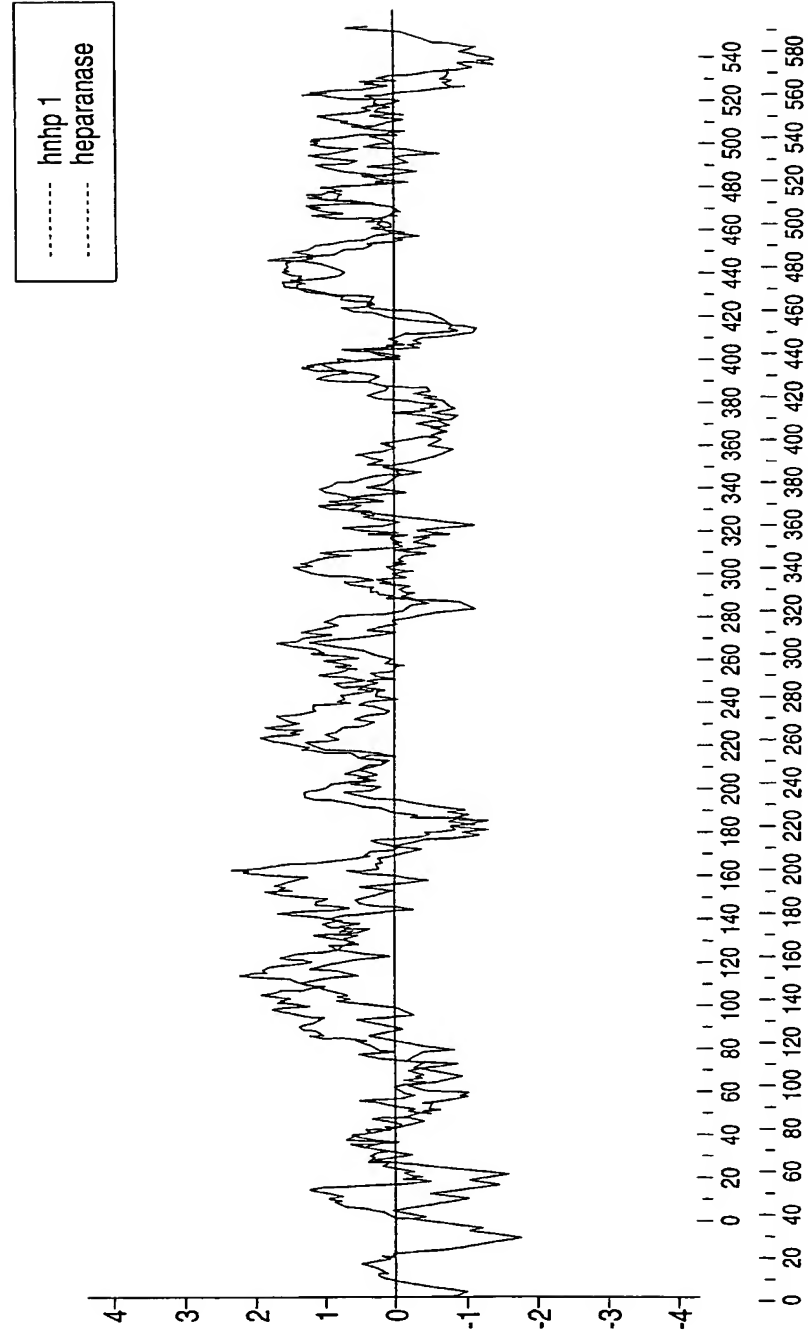
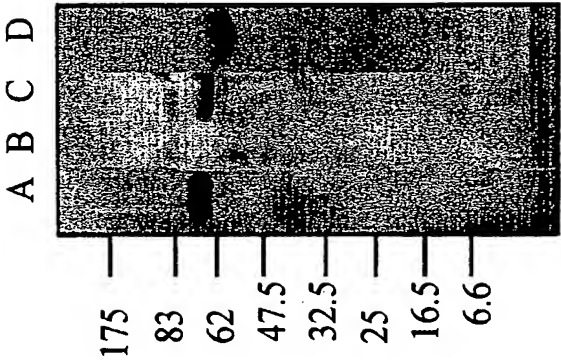


FIG.6

Figure 7





1  
SEQUENCE LISTING

- (1) GENERAL INFORMATION:
- (i) APPLICANT: Iris Pecker et al.
- (ii) TITLE OF INVENTION: POLYNUCLEOTIDES AND POLYPEPTIDES ENCODED THEREBY
- (iii) NUMBER OF SEQUENCES: 24
- (iv) CORRESPONDENCE ADDRESS:
- (A) ADDRESSEE: Sol Sheinbein c/o Anthony Castorina
- (B) STREET: 2001 Jefferson Davis Highway, Suite 207
- (C) CITY: Arlington
- (D) STATE: Virginia
- (E) COUNTRY: United States of America
- (F) ZIP: 22202
- (v) COMPUTER READABLE FORM:
- (A) MEDIUM TYPE: 1.44 megabyte, 3.5" microdisk
- (B) COMPUTER: Twinhead\* Slimnote-890TX
- (C) OPERATING SYSTEM: MS DOS version 6.2, Windows version 3.11
- (D) SOFTWARE: Word for Windows version 2.0 converted to an ASCII file
- (vi) CURRENT APPLICATION DATA:
- (A) APPLICATION NUMBER:
- (B) FILING DATE:
- (C) CLASSIFICATION:
- (vii) PRIOR APPLICATION DATA:
- (A) APPLICATION NUMBER: 60/140,801
- (B) FILING DATE: June 25, 1999
- (viii) ATTORNEY/AGENT INFORMATION:
- (A) NAME: Sheinbein, Sol
- (B) REGISTRATION NUMBER: 25,457
- (C) REFERENCE/DOCKET NUMBER: 20105
- (ix) TELECOMMUNICATION INFORMATION:
- (A) TELEPHONE: 972-3-6127676
- (B) TELEFAX: 972-3-6127575
- (C) TELEX:

## (2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 2060
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: double
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:
- |   |      |
|---|------|
| CGCTTAATTC TAGAAGAGGG ATTGAATGAG GGTGCTTTGT GCCTTCCTTG  | 50   |
| AAGCCATGCC CTCAGCAAC TCCCGCCCC CCGCGTGCCT AGCCCCGGGG    | 100  |
| GCTCTCTACT TGGCTCTGTT GCTCCATCTC TCCCTTTCCT CCCAGGCTGG  | 150  |
| AGACAGGAGA CCCTTGCTCG TAGACAGAGC TGCAGGTTTG AAGGAAAAGA  | 200  |
| CCCTGATTCT ACTTGATGTG AGCACCAAGA ACCCAGTCAG GACAGTCAAT  | 250  |
| GAGAACTTCC TCTCTCTGCA GCTGGATCCG TCCATCATTC ATGATGGCTG  | 300  |
| GCTCGATTTC CTAAGCTCCA AGCGCTTGGT GACCCCTGGCC CGGGGACTTT | 350  |
| CGCCCCGCTT TCTGCGCTTC GGGGGCAAAA GGACCGACTT CCTGCAGTTC  | 400  |
| CAGAACCTGA GGAACCCGGC GAAAAGCCGC GGGGGCCCCG GCCCGGATTA  | 450  |
| CTATCTCAAA AACTATGAGG ATGACATTGT TCGAAGTGAT GTTGCCCTAG  | 500  |
| ATAAACAGAA AGGCTGCAAG ATTGCCCAGC ACCCTGATGT TATGCTGGAG  | 550  |
| CTCCAAAGGG AGAAGGCAGC TCAGATGCAT CTGGTTCTTC TAAAGGAGCA  | 600  |
| ATTCTCCAAT ACTTACAGTA ATCTCATATT AACAGCCAGG TCTCTAGACA  | 650  |
| AACTTTATAA CTTTGCTGAT TGCTCTGGAC TCCACCTGAT ATTTGCTCTA  | 700  |
| AATGCACGTC GTCGTAATCC CAATACTCC TGGAACAGTT CTAGTGCCTT   | 750  |
| GAGTCTGTTG AAGTACAGCG CCAGCAAAA GTACAACATT TCTTGGGAAC   | 800  |
| TGGGTAATGA GCCAAATAAC TATCGGACCA TGCATGGCCG GGCAGTAAAT  | 850  |
| GGCAGCCAGT TGGGAAAGGA TTACATCCAG CTGAAGAGCC TGTTCAGCC   | 900  |
| CATCCGGATT TATTCCAGAG CCAGCTTATA TGGCCCTAAT ATTTGGCGGC  | 950  |
| CGAGGAAGAA TGTATCGGCC CTCCTAGATG GATTCATGAA GGTGGCAGGA  | 1000 |
| AGTACAGTAG ATGCAGTTAC CTGGCAACAT TGCTACATTG ATGGCCGGGT  | 1050 |
| GGTCAAGGTG ATGGACTTCC TGAAACTCG CCTGTTAGAC ACACTCTCTG   | 1100 |
| ACCAGATTAG GAAAATTGAG AAAGTGGTTA ATACATACAC TCCAGGAAAG  | 1150 |
| AAGATTTGGC TTGAAGGTGT GGTGACCACC TCAGCTGGAG GCACAAACAA  | 1200 |
| TCTATCCGAT TCCTATGCTG CAGGATTCTT ATGGTTGAAC ACTTTAGGAA  | 1250 |
| TGCTGGCCAA TCAGGGCATT GATGTCGTGA TACGGCACTC ATTTTTTGAC  | 1300 |
| CATGGATACA ATCACCCTGT GGACCAAGAT TTAAACCCAT TACCAGACTA  | 1350 |
| CTGGCTCTCT CTCCTCTACA AGCGCCTGAT CGGCCCAAAA GTCTTGGCTG  | 1400 |
| TGCATGTGGC TGGGCTCCAG CGGAAGCCAC GGCCTGGCCG AGTGATCCGG  | 1450 |
| GACAAACTAA GGATTTATGC TCACTGCACA AACCACCACA ACCACAACAA  | 1500 |
| CGTTCGTGGG TCCATTACAC TTTTATCAT CAACTTGCAT CGATCAAGAA   | 1550 |
| AGAAAATCAA GCTGGCTGGG ACTCTCAGAG ACAAGCTGGT TCACCAGTAC  | 1600 |
| CTGCTGCAGC CCTATGGGCA GGAGGGCCTA AAGTCCAAGT CAGTGAACCT  | 1650 |
| GAATGGCCAG CCCTTAGTGA TGGTGGACGA CGGGACCCTC CCAGAATTGA  | 1700 |
| AGCCCCGCCG CCTTCGGGCC GCGCGACAT TGGTCATCCC TCCAGTCACC   | 1750 |
| ATGGGCTTTT TTGTGGTCAA GAATGTCAAT GCTTTGGCCT GCCGCTACCG  | 1800 |

2

ATAAGCTATC CTCACACTCA TGGCTACCAG TGGGCCTGCT GGGCTGCTTC 1850  
 CACTCTCTCA CTCAGTAGT ATCCTCTGTT TTCAGACATC CTAGCAACCA 1900  
 GCCCCTGCTG CCCCATCCTG CTGGAATCAA CACAGACTTG CTCTCCAAAG 1950  
 AGACTAAATG TCATAGCGTG ATCTTAGCCT AGGTAGGCCA CATCCATCCC 2000  
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 TATAGACAA 2060

## (2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 2060  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

C	GCT	TAA	TTC	TAG	AAG	AGG	GAT	TGA	25
ATG	AGG	GTG	CTT	TGT	GCC	TTC	CCT	GAA	70
Met	Arg	Val	Leu	Cys	Ala	Phe	Pro	Glu	
									15
TCC	CGC	CCC	CCC	GCG	TGC	CTA	GCC	CCG	115
Ser	Arg	Pro	Pro	Ala	Cys	Leu	Ala	Pro	
									30
CTG	TTG	CTC	CAT	CTC	TCC	CTT	TCC	CAG	160
Leu	Leu	Leu	His	Leu	Ser	Leu	Ser	Gln	
									45
CCC	TTG	CCT	GTA	GAC	AGA	GCT	GCA	GGT	205
Pro	Leu	Pro	Val	Asp	Arg	Ala	Ala	Gly	
									60
ATT	CTA	CTT	GAT	GTG	AGC	ACC	AAG	AAC	250
Ile	Leu	Leu	Asp	Val	Ser	Thr	Lys	Asn	
									75
GAG	AAC	TTC	CTC	TCT	CTG	CAG	CTG	GAT	295
Glu	Asn	Phe	Leu	Ser	Leu	Gln	Leu	Asp	
									90
GGC	TGG	CTC	GAT	TTC	CTA	AGC	TCC	AAG	340
Gly	Trp	Leu	Asp	Phe	Leu	Ser	Ser	Lys	
									105
CGG	GGA	CTT	TCG	CCC	GCC	TTT	CTG	CGC	385
Arg	Gly	Leu	Ser	Pro	Ala	Phe	Leu	Arg	
									120
GAC	TTC	CTG	CAG	TTC	CAG	AAC	CTG	AGG	430
Asp	Phe	Leu	Gln	Phe	Gln	Asn	Leu	Arg	
									135
GGG	GGC	CCG	GGC	GAT	TAC	TAT	CTC	AAA	475
Gly	Gly	Pro	Gly	Pro	Asp	Tyr	Tyr	Leu	
									150
ATT	GTT	CGA	AGT	GAT	GTT	GCC	TTA	GAT	520
Ile	Val	Arg	Ser	Asp	Val	Ala	Leu	Asp	
									165
ATT	GCC	CAG	CAC	CCT	GAT	GTT	ATG	CTG	565
Ile	Ala	Gln	His	Pro	Asp	Val	Met	Leu	
									180
GCA	GCT	CAG	ATG	CAT	CTG	GTT	CTT	CTA	610
Ala	Ala	Gln	Met	His	Leu	Val	Leu	Leu	
									195
ACT	TAC	AGT	AAT	CTC	ATA	TTA	ACA	GCC	655
Thr	Tyr	Ser	Asn	Leu	Ile	Leu	Thr	Ala	
									210
TAT	AAC	TTT	GCT	GAT	TGC	TCT	GGA	CTC	700
Tyr	Asn	Phe	Ala	Asp	Cys	Ser	Gly	Leu	
									225
AAT	GCA	CTG	CGT	CGT	AAT	CCC	AAT	AAC	745
Asn	Ala	Leu	Arg	Arg	Asn	Pro	Asn	Asn	
									240
GCC	CTG	AGT	CTG	TTG	AAG	TAC	AGC	GCC	790
Ala	Leu	Ser	Leu	Leu	Lys	Tyr	Ser	Ala	
									255
TCT	TGG	GAA	CTG	GGT	AAT	GAG	CCA	AAT	835
Ser	Trp	Glu	Leu	Gly	Asn	Glu	Pro	Asn	
									270
GGC	CGG	GCA	GTA	AAT	GGC	AGC	CAG	TTG	880
Gly	Arg	Ala	Val	Asn	Gly	Ser	Gln	Leu	
									285
CTG	AAG	AGC	CTG	TTG	CAG	CCC	ATC	CGG	925
Leu	Lys	Ser	Leu	Leu	Gln	Pro	Ile	Arg	
									300
TTA	TAT	GGC	CCT	AAT	ATT	GGG	CGG	CCG	970
Leu	Tyr	Gly	Pro	Asn	Ile	Gly	Arg	Pro	
									315
CTC	CTA	GAT	GGA	TTC	ATG	AAG	GTG	GCA	1015
Leu	Leu	Asp	Gly	Phe	Met	Lys	Val	Ala	

3

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320      325      330
GTT ACC TGG CAA CAT TGC TAC ATT GAT GGC CGG GTG GTC AAG GTG 1060
Val Thr Trp Gln His Cys Tyr Ile Asp Gly Arg Val Val Lys Val
335      340      345
ATG GAC TTC CTG AAA ACT CGC CTG TTA GAC ACA CTC TCT GAC CAG 1105
Met Asp Phe Leu Lys Thr Arg Leu Leu Asp Thr Leu Ser Asp Gln
350      355      360
ATT AGG AAA ATT CAG AAA GTG GTT AAT ACA TAC ACT CCA GGA AAG 1150
Ile Arg Lys Ile Gln Lys Val Val Asn Thr Tyr Thr Pro Gly Lys
365      370      375
AAG ATT TGG CTT GAA GGT GTG GTG ACC ACC TCA GCT GGA GGC ACA 1195
Lys Ile Trp Leu Glu Gly Val Val Thr Thr Ser Ala Gly Gly Thr
380      385      390
AAC AAT CTA TCC GAT TCC TAT GCT GCA GGA TTC TTA TGG TTG AAC 1240
Asn Asn Leu Ser Asp Ser Tyr Ala Ala Gly Phe Leu Trp Leu Asn
395      400      405
ACT TTA GGA ATG CTG GCC AAT CAG GGC ATT GAT GTC GTG ATA CGG 1285
Thr Leu Gly Met Leu Ala Asn Gln Gly Ile Asp Val Val Ile Arg
410      415      420
CAC TCA TTT TTT GAC CAT GGA TAC AAT CAC CTC GTG GAC CAG AAT 1330
His Ser Phe Phe Asp His Gly Tyr Asn His Leu Val Asp Gln Asn
425      430      435
TTT AAC CCA TTA CCA GAC TAC TGG CTC TCT CTC CTC TAC AAG CGC 1375
Phe Asn Pro Leu Pro Asp Tyr Trp Leu Ser Leu Leu Tyr Lys Arg
440      445      450
CTG ATC GGC CCC AAA GTC TTG GCT GTG CAT GTG GCT GGG CTC CAG 1420
Leu Ile Gly Pro Lys Val Leu Ala Val His Val Ala Gly Leu Gln
455      460      465
CGG AAG CCA CGG CCT GGC CGA GTG ATC CGG GAC AAA CTA AGG ATT 1465
Arg Lys Pro Arg Pro Gly Arg Val Ile Arg Asp Lys Leu Arg Ile
470      475      480
TAT GCT CAC TGC ACA AAC CAC CAC AAC CAC AAC TAC GTT CGT GGG 1510
Tyr Ala His Cys Thr Asn His His Asn His Asn Tyr Val Arg Gly
485      490      495
TCC ATT ACA CTT TTT ATC ATC AAC TTG CAT CGA TCA AGA AAG AAA 1555
Ser Ile Thr Leu Phe Ile Ile Asn Leu His Arg Ser Arg Lys Lys
500      505      510
ATC AAG CTG GCT GGG ACT CTC AGA GAC AAG CTG GTT CAC CAG TAC 1600
Ile Lys Leu Ala Gly Thr Leu Arg Asp Lys Leu Val His Gln Tyr
515      520      525
CTG CTG CAG CCC TAT GGG CAG GAG GGC CTA AAG TCC AAG TCA GTG 1645
Leu Leu Gln Pro Tyr Gly Gln Glu Gly Leu Lys Ser Lys Ser Val
530      535      540
CAA CTG AAT GGC CAG CCC TTA GTG ATG GTG GAC GAC GGG ACC CTC 1690
Gln Leu Asn Gly Gln Pro Leu Val Met Val Asp Asp Gly Thr Leu
545      550      555
CCA GAA TTG AAG CCC CGC CCC CTT CGG GCC GGC CGG ACA TTG GTC 1735
Pro Glu Leu Lys Pro Arg Pro Leu Arg Ala Gly Arg Thr Leu Val
560      565      570
ATC CCT CCA GTC ACC ATG GGC TTT TTT GTG GTC AAG AAT GTC AAT 1780
Ile Pro Pro Val Thr Met Gly Phe Phe Val Val Lys Asn Val Asn
575      580      585
GCT TTG GCC TGC CGC TAC CGA TAA GCT ATC CTC ACA CTC ATG GCT 1825
Ala Leu Ala Cys Arg Tyr Arg
590
ACC AGT GGG CCT GCT GGG CTG CTT CCA CTC CTC CAC TCC AGT AGT 1870
ATC CTC TGT TTT CAG ACA TCC TAG CAA CCA GCC CCT GCT GCC CCA 1915
TCC TGC TGG AAT CAA CAC AGA CTT GCT CTC CAA AGA GAC TAA ATG 1960
TCA TAG CGT GAT CTT AGC CTA GGT AGG CCA CAT CCA TCC CAA AGG 2005
AAA ATG TAG ACA TCA CCT GTA CCT ATA TAA GGA TAA AGG CAT GTG 2050
TAT AGA GCA A 2060

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## (2) INFORMATION FOR SEQ ID NO:3:

## (i) SEQUENCE CHARACTERISTICS:

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(A) LENGTH: 592
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

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## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

```

Met Arg Val Leu Cys Ala Phe Pro Glu Ala Met Pro Ser Ser Asn
5
Ser Arg Pro Pro Ala Cys Leu Ala Pro Gly Ala Leu Tyr Leu Ala
20
Leu Leu Leu His Leu Ser Leu Ser Ser Gln Ala Gly Asp Arg Arg
35
Pro Leu Pro Val Asp Arg Ala Ala Gly Leu Lys Glu Lys Thr Leu
50
Ile Leu Leu Asp Val Ser Thr Lys Asn Pro Val Arg Thr Val Asn
65
Glu Asn Phe Leu Ser Leu Gln Leu Asp Pro Ser Ile Ile His Asp
75

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4

	80		85		90
Gly Trp Leu Asp Phe	Leu Ser Ser Lys Arg	Leu Val Thr Leu Ala			
	95		100		105
Arg Gly Leu Ser Pro	Ala Phe Leu Arg Phe	Gly Gly Lys Arg Thr			
	110		115		120
Asp Phe Leu Gln Phe	Gln Asn Leu Arg Asn	Pro Ala Lys Ser Arg			
	125		130		135
Gly Gly Pro Gly Pro	Asp Tyr Tyr Leu Lys	Asn Tyr Glu Asp Asp			
	140		145		150
Ile Val Arg Ser Asp	Val Ala Leu Asp Lys	Gln Lys Gly Cys Lys			
	155		160		165
Ile Ala Gln His Pro	Asp Val Met Leu Glu	Leu Gln Arg Glu Lys			
	170		175		180
Ala Ala Gln Met His	Leu Val Leu Leu Lys	Glu Gln Phe Ser Asn			
	185		190		195
Thr Tyr Ser Asn Leu	Ile Leu Thr Ala Arg	Ser Leu Asp Lys Leu			
	200		205		210
Tyr Asn Phe Ala Asp	Cys Ser Gly Leu His	Leu Ile Phe Ala Leu			
	215		220		225
Asn Ala Leu Arg Arg	Asn Pro Asn Asn Ser	Trp Asn Ser Ser Ser			
	230		235		240
Ala Leu Ser Leu Leu	Lys Tyr Ser Ala Ser	Lys Lys Tyr Asn Ile			
	245		250		255
Ser Trp Glu Leu Gly	Asn Glu Pro Asn Asn	Tyr Arg Thr Met His			
	260		265		270
Gly Arg Ala Val Asn	Gly Ser Gln Leu Gly	Lys Asp Tyr Ile Gln			
	275		280		285
Leu Lys Ser Leu Leu	Gln Pro Ile Arg Ile	Tyr Ser Arg Ala Ser			
	290		295		300
Leu Tyr Gly Pro Asn	Ile Gly Arg Pro Arg	Lys Asn Val Ile Ala			
	305		310		315
Leu Leu Asp Gly Phe	Met Lys Val Ala Gly	Ser Thr Val Asp Ala			
	320		325		330
Val Thr Trp Gln His	Cys Tyr Ile Asp Gly	Arg Val Val Lys Val			
	335		340		345
Met Asp Phe Leu Lys	Thr Arg Leu Leu Asp	Thr Leu Ser Asp Gln			
	350		355		360
Ile Arg Lys Ile Gln	Lys Val Val Asn Thr	Tyr Thr Pro Gly Lys			
	365		370		375
Lys Ile Trp Leu Glu	Gly Val Val Thr Thr	Ser Ala Gly Gly Thr			
	380		385		390
Asn Asn Leu Ser Asp	Ser Tyr Ala Ala Gly	Phe Leu Trp Leu Asn			
	395		400		405
Thr Leu Gly Met Leu	Ala Asn Gln Gly Ile	Asp Val Val Ile Arg			
	410		415		420
His Ser Phe Phe Asp	His Gly Tyr Asn His	Leu Val Asp Gln Asn			
	425		430		435
Phe Asn Pro Leu Pro	Asp Tyr Trp Leu Ser	Leu Leu Tyr Lys Arg			
	440		445		450
Leu Ile Gly Pro Lys	Val Leu Ala Val His	Val Ala Gly Leu Gln			
	455		460		465
Arg Lys Pro Arg Pro	Gly Arg Val Ile Arg	Asp Lys Leu Arg Ile			
	470		475		480
Tyr Ala His Cys Thr	Asn His His Asn His	Asn Tyr Val Arg Gly			
	485		490		495
Ser Ile Thr Leu Phe	Ile Ile Asn Leu His	Arg Ser Arg Lys Lys			
	500		505		510
Ile Lys Leu Ala Gly	Thr Leu Arg Asp Lys	Leu Val His Gln Tyr			
	515		520		525
Leu Leu Gln Pro Tyr	Gly Gln Glu Gly Leu	Lys Ser Lys Ser Val			
	530		535		540
Gln Leu Asn Gly Gln	Pro Leu Val Met Val	Asp Asp Gly Thr Leu			
	545		550		555
Pro Glu Leu Lys Pro	Arg Pro Leu Arg Ala	Gly Arg Thr Leu Val			
	560		565		570
Ile Pro Pro Val Thr	Met Gly Phe Phe Val	Val Lys Asn Val Asn			
	575		580		585
Ala Leu Ala Cys Arg	Tyr Arg				
	590				

## (2) INFORMATION FOR SEQ ID NO:4:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1898  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

CGCTTAATTC TAGAAGAGGG ATTGAATGAG GGTGCTTTGT GCCTTCCCTG	50
AAGCCATGCC CTCCAGCAAC TCCCGCCCCC CCGCGTGCCT AGCCCCGGGG	100
GCTCTCTACT TGGCTCTGTT GCTCCATCTC TCCCTTTCCT CCCAGGCTGG	150

5

AGACAGGAGA CCCTTGCCTG TAGACAGAGC TGCAGGTTTG AAGGAAAAGA 200  
 CCCTGATTCT ACTTGATGTG AGCACCAAGA ACCCAGTCAG GACAGTCAAT 250  
 GAGAACTTCC TCTCTCTGCA GCTGGATCCG TCCATCATTC ATGATGGCTG 300  
 GCTCGATTTC CTAAGCTCCA AGCGCTTGGT GACCCTGGCC CGGGGACTTT 350  
 CGCCCGCCTT TCTGCGCTTC GGGGGCAAAA GGACCGACTT CCTGCAGTTC 400  
 CAGAACCTGA GGAACCCGGC GAAAGCCGC GGGGGCCCGG GCCCGGATTA 450  
 CTATCTCAAA AACTATGAGG ATGCCAGGTC TCTAGACAAA CTTTATAACT 500  
 TTGCTGATTG CTCTGGACTC CACCTGATAT TTGCTCTAAA TGCACTGCGT 550  
 CGTAATCCCA ATAACCTCTG GAACAGTTCT AGTGCCCTGA GTCTGTTGAA 600  
 GTACAGCGCC AGCAAAAAGT ACAACATTTC TTGGGAAC TGTAATGAGC 650  
 CAAATAAGTA TCGGACCATG CATGGCCGGG CAGTAAATGG CAGCCAGTTG 700  
 GGAAGAGGAT ACATCCAGCT GAAGAGCCTG TTGCAGCCCA TCCGGATTTA 750  
 TTCCAGAGCC AGCTTATATG GCCCTAATAT TGGGCGGCCG AGGAAGAATG 800  
 TCATCGCCCT CCTAGATGGA TTCATGAAGG TGGCAGGAAG TACAGTAGAT 850  
 GCAGTTACCT GGCACATTG CTACATTGAT GGCCTGGTGG TCAAGGTGAT 900  
 GGACTTCCTG AAAACTCGCC TGTAGACAC ACTCTCTGAC CAGATTAGGA 950  
 AAATTCAGAA AGTGGTTAAT ACATACACTC CAGGAAAAGAA GATTGGCTT 1000  
 GAAGGTGTGG TGACCACCTC AGCTGGAGGC ACAACAATC TATCCGATTC 1050  
 CTATGCTGCA GGATTCTTAT GGTGAACAC TTTAGGAATG CTGGCCAATC 1100  
 AGGGCATTGA TGTGCTGATA CGGCACTCAT TTTTGTACCA TGGATACAAT 1150  
 CACCTCGTGG ACCAGAATTT TAACCCATTA CCAGACTACT GGCCTCTCTG 1200  
 CCTCTACAAG CGCTGATCG GCCCAAAGT CTTGGCTGTG CATGTGGCTG 1250  
 GGCTCCAGCG GAAGCCACGG CCTGGCCGAG TGATCCGGGA CAAACTAAGG 1300  
 ATTTATGCTC ACTGCACAAA CCACCACAAC CACAACACTG TTCGTGGGTC 1350  
 CATTACACTT TTTATCATCA ACTTGCATCG ATCAAGAAAG AAAATCAAGC 1400  
 TGGCTGGGAC TCTCAGAGAC AAGCTGGTTC ACCAGTACCT GCTGCAGCCC 1450  
 TATGGGCAGG AGGCGCTAAA GTCCAAGTCA GTGCAACTGA ATGGCCAGCC 1500  
 CTTAGTGATG GTGGACGACG GGACCCCTCC AGAATTGAAG CCCCGCCCCC 1550  
 TTCGGGCCGG CCGGACATTG GTCATCCCTC CAGTCACCAT GGGCTTTTTT 1600  
 GTGGTCAAGA ATGTCAATGC TTTGGCCTGC CGTACCAGT AAGCTATCCT 1650  
 CACACTCATG GCTACCAAGT GGCCTGCTGG GCTGCTTCCA CTCCTCCACT 1700  
 CCAGTAGTAT CCTCTGTTT CAGACATCCT AGCAACCAAGC CCCTGCTGCC 1750  
 CCATCTGCTT GGAATCAACA CAGACTTGCT CTCCAAGAG ACTAAATGTC 1800  
 ATAGCGTGAT CTTAGCCTAG GTAGGCCACA TCCATCCCAA AGGAAAATGT 1850  
 AGACATCACC TGTACCTATA TAAGGATAAA GGCATGTGTA TAGAGCAA 1898

- 2) INFORMATION FOR SEQ ID NO:5:
- (i) SEQUENCE CHARACTERISTICS:
- (A) LENGTH: 538
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Met Arg Val Leu Cys Ala Phe Pro Glu Ala Met Pro Ser Ser Asn  
 5 10  
 Ser Arg Pro Pro Ala Cys Leu Ala Pro Gly Ala Leu Tyr Leu Ala  
 20 25 30  
 Leu Leu Leu His Leu Ser Leu Ser Ser Gln Ala Gly Asp Arg Arg  
 35 40 45  
 Pro Leu Pro Val Asp Arg Ala Ala Gly Leu Lys Glu Lys Thr Leu  
 50 55 60  
 Ile Leu Leu Asp Val Ser Thr Lys Asn Pro Val Arg Thr Val Asn  
 65 70 75  
 Glu Asn Phe Leu Ser Leu Gln Leu Asp Pro Ser Ile Ile His Asp  
 80 85 90  
 Gly Trp Leu Asp Phe Leu Ser Ser Lys Arg Leu Val Thr Leu Ala  
 95 100 105  
 Arg Gly Leu Ser Pro Ala Phe Leu Arg Phe Gly Gly Lys Arg Thr  
 110 115 120  
 Asp Phe Leu Gln Phe Gln Asn Leu Arg Asn Pro Ala Lys Ser Arg  
 125 130 135  
 Gly Gly Pro Gly Pro Asp Tyr Tyr Leu Lys Asn Tyr Glu Asp Ala  
 140 145 150  
 Arg Ser Leu Asp Lys Leu Tyr Asn Phe Ala Asp Cys Ser Gly Leu  
 155 160 165  
 His Leu Ile Phe Ala Leu Asn Ala Leu Arg Arg Asn Pro Asn Asn  
 170 175 180  
 Ser Trp Asn Ser Ser Ser Ala Leu Ser Leu Leu Lys Tyr Ser Ala  
 185 190 195  
 Ser Lys Lys Tyr Asn Ile Ser Trp Glu Leu Gly Asn Glu Pro Asn  
 200 205 210  
 Asn Tyr Arg Thr Met His Gly Arg Ala Val Asn Gly Ser Gln Leu  
 215 220 225  
 Gly Lys Asp Tyr Ile Gln Leu Lys Ser Leu Leu Gln Pro Ile Arg  
 230 235 240  
 Ile Tyr Ser Arg Ala Ser Leu Tyr Gly Pro Asn Ile Gly Arg Pro  
 245 250 255  
 Arg Lys Asn Val Ile Ala Leu Leu Asp Gly Phe Met Lys Val Ala

				260						265				270
Gly	Ser	Thr	Val	Asp	Ala	Val	Thr	Trp	Gln	His	Cys	Tyr	Ile	Asp
				275					280					285
Gly	Arg	Val	Val	Lys	Val	Met	Asp	Phe	Leu	Lys	Thr	Arg	Leu	Leu
				290					295					300
Asp	Thr	Leu	Ser	Ala	Gln	Ile	Arg	Lys	Ile	Gln	Lys	Val	Val	Asn
				305					310					315
Thr	Tyr	Thr	Pro	Gly	Lys	Lys	Ile	Trp	Leu	Glu	Gly	Val	Val	Thr
				320					325					330
Thr	Ser	Ala	Gly	Gly	Thr	Asn	Asn	Leu	Ser	Asp	Ser	Tyr	Ala	Ala
				335					340					345
Gly	Phe	Leu	Trp	Leu	Asn	Thr	Leu	Gly	Met	Leu	Ala	Asn	Gln	Gly
				350					355					360
Ile	Asp	Val	Val	Ile	Arg	His	Ser	Phe	Phe	Asp	His	Gly	Tyr	Asn
				365					370					375
His	Leu	Val	Asp	Gln	Asn	Phe	Asn	Pro	Leu	Pro	Asp	Tyr	Trp	Leu
				380					385					390
Ser	Leu	Leu	Tyr	Lys	Arg	Leu	Ile	Gly	Pro	Lys	Val	Leu	Ala	Val
				395					400					405
His	Val	Ala	Gly	Leu	Gln	Arg	Lys	Pro	Arg	Pro	Gly	Arg	Val	Ile
				410					415					420
Arg	Asp	Lys	Leu	Arg	Ile	Tyr	Ala	His	Cys	Thr	Asn	His	His	Asn
				425					430					435
His	Asn	Tyr	Val	Arg	Gly	Ser	Ile	Thr	Leu	Phe	Ile	Ile	Asn	Leu
				440					445					450
His	Arg	Ser	Arg	Lys	Lys	Ile	Lys	Leu	Ala	Gly	Thr	Leu	Arg	Asp
				455					460					465
Lys	Leu	Val	His	Gln	Tyr	Leu	Leu	Gln	Pro	Tyr	Gly	Gln	Glu	Gly
				470					475					480
Leu	Lys	Ser	Lys	Ser	Val	Gln	Leu	Asn	Gly	Gln	Pro	Leu	Val	Met
				485					490					495
Val	Asp	Asp	Gly	Thr	Leu	Pro	Glu	Leu	Lys	Pro	Arg	Pro	Leu	Arg
				500					505					510
Ala	Gly	Arg	Thr	Leu	Val	Ile	Pro	Pro	Val	Thr	Met	Gly	Phe	Phe
				515					520					525
Val	Val	Lys	Asn	Val	Asn	Ala	Leu	Ala	Cys	Arg	Tyr	Arg		
				530					535					

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 1724  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GCCTTAATTC	TAGAGAGGG	ATTGAATGAG	GGTGCTTTGT	GCCTTCCCTG	50
AAGCTACATGCC	CTCCAGCAAC	TCCCGCCCCC	CCGCGTGCCCT	AGCCCCGGGG	100
GCCTCTTACT	TGGCTCTGTT	TGCTCATATC	TCCCTTTCCCT	CCACCGCTGG	150
AGACAGGAGA	CCCTTGCCGT	TAGACAGAGC	TGCGAGTTTG	AGGAAAAGA	200
CCCTGATTCT	ACTTGATGTG	AGACACAAGA	ACCCAGTCAG	GACAGCTCAAT	250
GAGAACTTCC	TCTCTCTGCA	TGCTGGATCCG	TCCATCATTC	ATGATGGCTG	300
CTGCGATTTC	CTAAGCTCCA	AGCGCTTGGT	GACCCGTGCC	CGGGGACTTT	350
CGCCCGCCTT	TCTCGGCTTC	GGGGGCAAAA	GGACCGACTT	CTCTGCACTT	400
CAGAACTCTGA	GGAAACCCGG	GAAAAGCCCG	GGGGGCGCCG	CGCCGGATTA	450
CTATCTCAAA	AACTATGAGG	ATGAGGCCAA	TAACTATCGG	ACCATGCATG	500
CGCCGGCAGT	AAATGGGACG	CAGTTGGGAA	AGGATTATAC	CCAGCTGAAG	550
AGCCTGTGTG	AGCCCATCCG	GATTTATTCC	AGAGACGACT	TATATGGCCC	600
TAAATTGGGG	CGCCCGAGGA	AGAAATGTCAT	CGCCCTCTCTA	GATGGATTCA	650
TGAGGTTGGG	AGGAAGTACA	GTAGATGCAG	TTACCTGGCA	ACATTGCTAC	700
ATTGATGGCC	GGGTGGTCAA	GGTGATGAAC	TCGTGAAGAA	CTTCACTGAT	750
AGACCACTC	TGTGCACAGA	TTAGGAAAT	TCAGAAAGT	GTTAACTCAT	800
ACACTCCAGG	AAAGAAGATT	TGGCTTGAAG	GTGTGGTGAC	CACCTCAGCT	850
GAGGACACAA	ACAATCTATC	CGATTCCCAT	GCTCGAGGAT	TCTTATGGTT	900
GAACCTCTTA	GGAAATGCTG	CCAAATCAGG	CATTGATGTC	GTGATACGGC	950
ACTCATTTTT	TGACCATGGA	TACAATCACC	TCTGTGACCA	GAAITTTTAA	1000
CCATTACCAG	ACTACTGGCT	CTCTCTCTCT	TCAAGCGCC	TGATCGGCC	1050
CAAAGCTCTG	GCTGTGCATG	TGGCTGGGCT	CACGCGGAAG	CCACGGCCCT	1100
CGCGAGTAGT	CGGGGACAA	CTAAGGATTT	ATGCTCACTG	CACAAACCAC	1150
CACAACCACA	ACTACGTTCC	TGGGTCCATT	ACACTTTTTA	TCATCAACTT	1200
GCATCGATCA	AGAAAGAAAA	TCAAGCTGGC	TGGGACTCTC	AGAGACAAGC	1250
TGTTTCACCA	GTACTCTGCT	CAGCCGATG	GGGAGAGGG	CTTAAGATCC	1300
AAGTCAGTGC	ACTGTAATGT	CCAGCCCTTA	TGTAGTGGTG	ACAGCGGGAC	1350
CTTCCCGAGA	TTGAAGCCCC	CGCCCTCTCG	GGCCGCGCCG	ACATTGGCTA	1400
TCCCTCCAGT	CACCATGGGC	TTTTTTGTGG	TCAAGAATGT	CAATGCTTTG	1450
GCCTTGCCTG	ACCGATAAGC	TATCCTCACA	TCATGGCTA	CGCTGGGCC	1500
TGCTGGGCTG	CTTCCACTCC	TCCACTCCAG	TAGTATCTCT	TGTTTTCAGA	1550
CATCTTAGCA	ACAGAGCCCT	GCTGCCCCAT	CTGCTCTGGA	GCACACAGA	1600
CTTGCTCTCC	AAGAGACTA	ATGTGCATAG	CTGTACTTTA	TCCTAGGTAG	1650
GCCAACTCCA	TCCCAAAGGA	AAATGTAGAC	ATCACCTGTA	CCATATAAAG	1700
GATAAAGGCA	TGTGTATAGA	GCAA			1720

## (2) INFORMATION FOR SEQ ID NO:7:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 480  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

```

Met Arg Val Leu Cys Ala Phe Pro Glu Ala Met Pro Ser Ser Asn
      5      10      15
Ser Arg Pro Pro Ala Cys Leu Ala Pro Gly Ala Leu Tyr Leu Ala
      20      25      30
Leu Leu Leu His Leu Ser Leu Ser Ser Gln Ala Gly Asp Arg Arg
      35      40      45
Pro Leu Pro Val Asp Arg Ala Ala Gly Leu Lys Glu Lys Thr Leu
      50      55      60
Ile Leu Leu Asp Val Ser Thr Lys Asn Pro Val Arg Thr Val Asn
      65      70      75
Glu Asn Phe Leu Ser Leu Gln Leu Asp Pro Ser Ile Ile His Asp
      80      85      90
Gly Trp Leu Asp Phe Leu Ser Ser Lys Arg Leu Val Thr Leu Ala
      95     100     105
Arg Gly Leu Ser Pro Ala Phe Leu Arg Phe Gly Gly Lys Arg Thr
     110     115     120
Asp Phe Leu Gln Phe Gln Asn Leu Arg Asn Pro Ala Lys Ser Arg
     125     130     135
Gly Gly Pro Gly Pro Asp Tyr Tyr Leu Lys Asn Tyr Glu Asp Glu
     140     145     150
Pro Asn Asn Tyr Arg Thr Met His Gly Arg Ala Val Asn Gly Ser
     155     160     165
Gln Leu Gly Lys Asp Tyr Ile Gln Leu Lys Ser Leu Leu Gln Pro
     170     175     180
Ile Arg Ile Tyr Ser Arg Ala Ser Leu Tyr Gly Pro Asn Ile Gly
     185     190     195
Arg Pro Arg Lys Asn Val Ile Ala Leu Leu Asp Gly Phe Met Lys
     200     205     210
Val Ala Gly Ser Thr Val Asp Ala Val Thr Trp Gln His Cys Tyr
     215     220     225
Ile Asp Gly Arg Val Val Lys Val Met Asp Phe Leu Lys Thr Arg
     230     235     240
Leu Leu Asp Thr Leu Ser Asp Gln Ile Arg Lys Ile Gln Lys Val
     245     250     255
Val Asn Thr Tyr Thr Pro Gly Lys Lys Ile Trp Leu Glu Gly Val
     260     265     270
Val Thr Thr Ser Ala Gly Gly Thr Asn Asn Leu Ser Asp Ser Tyr
     275     280     285
Ala Ala Gly Phe Leu Trp Leu Asn Thr Leu Gly Met Leu Ala Asn
     290     295     300
Gln Gly Ile Asp Val Val Ile Arg His Ser Phe Phe Asp His Gly
     305     310     315
Tyr Asn His Leu Val Asp Gln Asn Phe Asn Pro Leu Pro Asp Tyr
     320     325     330
Trp Leu Ser Leu Leu Tyr Lys Arg Leu Ile Gly Pro Lys Val Leu
     335     340     345
Ala Val His Val Ala Gly Leu Gln Arg Lys Pro Arg Pro Gly Arg
     350     355     360
Val Ile Arg Asp Lys Leu Arg Ile Tyr Ala His Cys Thr Asn His
     365     370     375
His Asn His Asn Tyr Val Arg Gly Ser Ile Thr Leu Phe Ile Ile
     380     385     390
Asn Leu His Arg Ser Arg Lys Lys Ile Lys Leu Ala Gly Thr Leu
     395     400     405
Arg Asp Lys Leu Val His Gln Tyr Leu Leu Gln Pro Tyr Gly Gln
     410     415     420
Glu Gly Leu Lys Ser Lys Ser Val Gln Leu Asn Gly Gln Pro Leu
     425     430     435
Val Met Val Asp Asp Gly Thr Leu Pro Glu Leu Lys Pro Arg Pro
     440     445     450
Leu Arg Ala Gly Arg Thr Leu Val Ile Pro Pro Val Thr Met Gly
     455     460     465
Phe Phe Val Val Lys Asn Val Asn Ala Leu Ala Cys Arg Tyr Arg
     470     475     480

```

## (2) INFORMATION FOR SEQ ID NO:8:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 351  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

8

GTTCGGCAGA GGATCATGTC TGATGTACAG AGACATTGTC CGGAGTGATG 50  
 TTGCCTTGGA CAAGCAGAAA GGCTGTAAGA TTGGCCAGCA CCTGTATGTC 100  
 ATGCTGGAGC TCCAGAGAGA GAAGGCATCC AGACTGTCTG GTTCTTCTGA 150  
 AGGAGCAATA CTCCAATACT TACAGTAACC TCATATTAAC AGGTCTCTAG 200  
 ACAAACCTTA TAACTTTGCT GATTGCTCTG GACTCCACCT GATATTGCT 250  
 CTAATGCAC TGCCTCGTAA TCCAATAAC TCCTGGAACA GTTCTAGTGC 300  
 CCTGAGCCTG TTGAAGTACA GTGCCAGCAA AAAGTACAAC ATTTCTTGGG 350  
 A 351

## (2) INFORMATION FOR SEQ ID NO:9:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 543  
 (B) TYPE: amino acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Met Leu Leu Arg Ser Lys Pro Ala Leu Pro Pro Pro Leu Met Leu Leu  
 5 10 15  
 Leu Leu Gly Pro Leu Gly Pro Leu Ser Pro Gly Ala Leu Pro Arg Pro  
 20 25 30  
 Ala Gln Ala Gln Asp Val Val Asp Leu Asp Phe Phe Thr Gln Glu Pro  
 35 40 45  
 Leu His Leu Val Ser Pro Ser Phe Leu Ser Val Thr Ile Asp Ala Asn  
 50 55 60  
 Leu Ala Thr Asp Pro Arg Phe Leu Ile Leu Leu Gly Ser Pro Lys Leu  
 65 70 75  
 Arg Thr Leu Ala Arg Gly Leu Ser Pro Ala Tyr Leu Arg Phe Gly Gly  
 85 90 95  
 Thr Lys Thr Asp Phe Leu Ile Phe Asp Pro Lys Lys Glu Ser Thr Phe  
 100 105 110  
 Glu Glu Arg Ser Tyr Trp Gln Ser Gln Val Asn Gln Asp Ile Cys Lys  
 115 120 125  
 Tyr Gly Ser Ile Pro Pro Asp Val Glu Glu Lys Leu Arg Leu Glu Trp  
 130 135 140  
 Pro Tyr Gln Glu Gln Leu Leu Leu Arg Glu His Tyr Gln Lys Lys Phe  
 145 150 155  
 Lys Asn Ser Thr Tyr Ser Arg Ser Ser Val Asp Val Leu Tyr Thr Phe  
 165 170 175  
 Ala Asn Cys Ser Gly Leu Asp Leu Ile Phe Gly Leu Asn Ala Leu Leu  
 180 185 190  
 Arg Thr Ala Asp Leu Gln Trp Asn Ser Ser Asn Ala Gln Leu Leu Leu  
 195 200 205  
 Asp Tyr Cys Ser Ser Lys Gly Tyr Asn Ile Ser Trp Glu Leu Gly Asn  
 210 215 220  
 Glu Pro Asn Ser Phe Leu Lys Lys Ala Asp Ile Phe Ile Asn Gly Ser  
 225 230 235  
 Gln Leu Gly Glu Asp Tyr Ile Gln Leu His Lys Leu Leu Arg Lys Ser  
 245 250 255  
 Thr Phe Lys Asn Ala Lys Leu Tyr Gly Pro Asp Val Gly Gln Pro Arg  
 260 265 270  
 Arg Lys Thr Ala Lys Met Leu Lys Ser Phe Leu Lys Ala Gly Gly Glu  
 275 280 285  
 Val Ile Asp Ser Val Thr Trp His His Tyr Tyr Leu Asn Gly Arg Thr  
 290 295 300  
 Ala Thr Arg Glu Asp Phe Leu Asn Pro Asp Val Leu Asp Ile Phe Ile  
 305 310 315  
 Ser Ser Val Gln Lys Val Phe Gln Val Val Glu Ser Thr Arg Pro Gly  
 325 330 335  
 Lys Lys Val Trp Leu Gly Glu Thr Ser Ser Ala Tyr Gly Gly Ala Ala  
 340 345 350  
 Pro Leu Leu Ser Asp Thr Phe Ala Ala Gly Phe Met Trp Leu Asp Lys  
 355 360 365  
 Leu Gly Leu Ser Ala Arg Met Gly Ile Glu Val Val Met Arg Gln Val  
 370 375 380  
 Phe Phe Gly Ala Gly Asn Tyr His Leu Val Asp Glu Asn Phe Asp Pro  
 385 390 395  
 Leu Pro Asp Tyr Trp Leu Ser Leu Leu Phe Lys Lys Leu Val Gly Thr  
 405 410 415  
 Lys Val Leu Met Ala Ser Val Gln Gly Ser Lys Arg Arg Lys Leu Arg  
 420 425 430  
 Val Tyr Leu His Cys Thr Asn Thr Asp Asn Pro Arg Tyr Lys Glu Gly  
 435 440 445  
 Asp Leu Thr Leu Tyr Ala Ile Asn Leu His Asn Val Thr Lys Tyr Leu  
 450 455 460  
 Arg Leu Pro Tyr Pro Phe Ser Asn Lys Gln Val Asp Lys Tyr Leu Leu  
 465 470 475  
 Arg Pro Leu Gly Pro His Gly Leu Leu Ser Lys Ser Val Gln Leu Asn  
 485 490 495  
 Gly Leu Thr Leu Lys Met Val Asp Asp Gln Thr Leu Pro Pro Leu Met  
 500 505 510



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Glu Lys Pro Leu Arg Pro Gly Ser Ser Leu Gly Leu Pro Ala Phe Ser  
515 520 525  
Tyr Ser Phe Phe Val Ile Arg Asn Ala Lys Val Ala Ala Cys Ile  
530 535 540

(2) INFORMATION FOR SEQ ID NO:10:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:  
GGAGAGCAAG TCTGTGTTGA TTC 23

(2) INFORMATION FOR SEQ ID NO:11:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:  
CACTGGTAGC CATGAGTGTG AG 22

(2) INFORMATION FOR SEQ ID NO:12:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 22  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:  
TTGGTCATCC CTCCAGTCAC CA 22

(2) INFORMATION FOR SEQ ID NO:13:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:  
Asp Glu

(2) INFORMATION FOR SEQ ID NO:14:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 23  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:  
CTTGCCCTGTA GACAGAGCTG CAG 23

(2) INFORMATION FOR SEQ ID NO:15:  
(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 2396  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear  
(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:  
TTTCTAGTTG CTTTATGCCA ATGTCGGATC AGGTTTTC AAGCGACAAAG 50  
AGATACTGAG ATCCTGGGCA GAGACATCC TAGCTCGGTC AGATTGGGTC 100  
AGGCTCAAGT GACCAAGTGC TTAAGGCAGA AGGGAGTCGG GGTAGGGTCT 150  
GGCTGAACCC TCAACCGGGG CTTTAACTC AGGGTCTAGT CCTGGCGCCA 200  
AATGGATGGG ACCTAGAAAA GGTGACAGAG TCGCGCAGGAC ACCAGGAAGC 250  
TGGTCCCAAC CCTGCGCGGC TCCCGGGCGC TCCTCCCCCA GGCTCCGAG 300  
GATCTTGAT TCTGGCCACC TCCGCACCTT TTGGATGGGT GTGGATGATT 350  
TCAAAAGTGG ACGTGACCGC GGCGGAGGGG AAAGCCAGCA CGGAAATGAA 400  
AGAGAGCGAG GAGGGGAGGG CGGGGAGGGG AGGGCGCTAG GGAGGGACTC 450  
CCGGGAGGGG TGGGAGGGAT GGAGCGCTGT GGGAGGGTAC TGAGTCCTGG 500  
CGCCAGAGGC GAAGCAGGAC CGGTTGCAGG GGGCTTGAGC CAGCGCGCCG 550  
GCTGCCCCAG CTCTCCCGGC AGCGGGCGGT CCAGCCAGGT GGGATGCTGA 600  
GGCTGTGCT GCTGTGGCTC TGGGGGCCGC TCGGTGCCCT GGCCAGGGC 650  
GCCCCCGGGG GGACCGCGCC GACCGACGAC GTGGTAGACT TGGAGTTTA 700  
CACCAAGCGG CCGCTCCGAA GCGTGAGTCC CTCGTTCTCTG TCCATCACA 750  
TCGACGCCAG CTTGGCCACC GACCCGCGCT TCCTCACCTT CCTGGGCTCT 800  
CCAAGGCTCC GTGCTCTGGC TAGAGGCTTA TCTCTGCACT ACTTGAGATT 850  
TGGCGGCACA AAGACTGACT TCCTTATTTT TGATCCGGAC AAGGAACCGA 900  
CTTCCGAAGA AAGAAGTTAC TGGAAATCTC AAGTCAACCA TGATATTTGC 950  
AGGTCTGAGC CGGTCTCTGC TGCGGTGTTG AGGAACTCC AGGTGGAATG 1000  
GCCCTTCCAG GAGCTGTTGC TGCTCCGAGA GCAGTACCAA AAGGAGTTCA 1050

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AGAACAGCAC CTACTCAAGA AGCTCAGTGG ACATGCTCTA CAGTTTGGCC 1100  
 AAGTGCTCGG GGTTAGACCT GATCTTTGGT CTAAATGCGT TACTACGAAC 1150  
 CCCAGACTTA CGGTGGAACA GctCCAACGC CCAGCTTCTC CTTGACTACT 1200  
 GCTCTTCCAA GGGTTATAAC ATcTCCTGGG AACTGGGCAA TGAGCCCAAC 1250  
 AGTTTcTGGa AGAAAGCTCA CATTCCTATC GATGGGTTGC AGTTAGGAGA 1300  
 AGACTTTGTG GAGTTGCATA AACTTcTACA AAGGTcAGCT TTCCAAATG 1350  
 CAAAACCTTA TGGTCCTGAC ATCGGTCAGC CTGAGGGAA GACAGTTAAA 1400  
 CTGCTGAGGA GTTTCCTGAA GGCTGGCGGA GAAGTGATCG ACTCTCTTAC 1450  
 ATGGCATCAC TATTACTTGA ATGGACGCAT CGCTACCAAA GAAGATTTTC 1500  
 TGAGCTCTGA TGGCTGAGAC ACTTTTATTc TCTCTGTGCA AAAAAATTCTG 1550  
 AAGGTCACTA AAGAGATCAC ACCTGGCAAG AAGGTCTGGT TGGGAGAGAC 1600  
 GAGCTCAGCT TACGGTGGCG GTGCACCCTT GCTGTCCAAC ACCTTTGCAG 1650  
 CTGGCTTTAT GTGGCTGGAT AAATTGGGCC TGTCAGCCCA GATGGGCATA 1700  
 GAAGTCGTGA TGAGCGAGGT GTTCTTCGGA GCAGGCAACT ACCACTTAGT 1750  
 GGATGAAAAC TTTGAGCCTT TACCTGATTA CTGGCTCTCT CTCTGTTCa 1800  
 AGAAACTGGT AGGTCCCAGG GTGTTACTGT CAAGAGTGAA AGGCCAGAC 1850  
 AGGAGCAAAc TCCGAGTGTA TCTCCACTGC ACTAACGTCT ATCACCACG 1900  
 ATATCAGGAA GGAGATCTAA CTCTGTATGT CCTGAACCTC CATAATGTCA 1950  
 CCAAGCACTT GAAGGTACCG CCTCCGTTGT TCAGGAAACC AGTGATACG 2000  
 TACCTTCTGA AGCCTTCGGG GCCGGATGGA TTACTTTCCA AATCTGTCCA 2050  
 ACTGAACGGT CAAATTCTGA AGATGGTGGA TGAGCAGACC CTGCCAGCTT 2100  
 TGACAGAAAA ACCTCTCCCC GCAGGAAGTG CACTAAGCCT GCCTGCCTT 2150  
 TCCTATGGTT TTTTGTGCAT AAGAAATGCC AAAATCGCTG CTGTATATG 2200  
 AAAATAAAAG GCATACGGTA CCCCTGAGAC AAAAGCCGAG GGGGGTGTTA 2250  
 TTCATAAAAC AAACCCCTAG TTTAGGAGGC CACCTCCTTG CCGAGTTCCA 2300  
 GAGCTTCGGG AGGTGGGGT ACACCTCAGT ATTACATTCA GTGTGGTGTT 2350  
 CTCTTAAGA AGAATACTGC AGGTGGTGAC AGTTAATAGC ACTGTG 2396

(2) INFORMATION FOR SEQ ID NO:16:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 22  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:  
 GAGCAGCCAG GTGAGCCCA GA 22

(2) INFORMATION FOR SEQ ID NO:17:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 24  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:  
 TCAGATGCAA GCAGCAACTT TGGC 24

(2) INFORMATION FOR SEQ ID NO:18:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 21  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:  
 CACCTTGATG TCATGCTGGA G 21

(2) INFORMATION FOR SEQ ID NO:19:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 23  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:  
 CATCTAGGAG AGCAATGACG TTC 23

(2) INFORMATION FOR SEQ ID NO:20:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 27  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear  
 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:  
 CCATCCTAAT ACAGCTCACT ATAGGGC 27

(2) INFORMATION FOR SEQ ID NO:21:  
 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 23  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: single  
 (D) TOPOLOGY: linear

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(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:  
ACTCACTATA GGGCTCGAGC GGC 23

## (2) INFORMATION FOR SEQ ID NO:22:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 15  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:  
TTTTTTTTTT TTTT 15

## (2) INFORMATION FOR SEQ ID NO:23:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 560  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23

GGCAGGAGGC	TAGTGGAGAG	ACTGACAAGC	AGTCAGCTCA	GCGGTCACAA	50
TACTGTGTGA	CAGGAGCTGA	GATCCAAGAA	GTACTGGGTC	CTGTGGGAGC	100
ACCCCTGACT	TGAAGGACAA	GTCAGTGCAA	CTGAATGGCC	AGCCCTTAGT	150
GATGGTGGAC	GACGGGACCC	TCCCAGAATT	GAAGCCCCGC	CCCTTCGGG	200
CCGGCCGGAC	ATTGGTCATC	CCTCCAGTCA	CCATGGGCTT	TTTTGTGGTC	250
AAGAATGTCA	ATGCTTTGGC	CTGCCGCTAC	CGATAAGCTA	TCCTCACACT	300
CATGGCTACC	AGTGGGCCCTG	CTGGGCTGCT	TCCACTCCTC	CACTCCAGTA	350
GTATCCTCTG	TTTTCAGACA	TCCTAGCAAC	CAGCCCCTGC	TGCCCCATCC	400
TGCTGGAATC	AACACAGACT	TGCTCTCCAA	AGAGACTAAA	TGTCATAGCG	450
TGATCTTAGC	CTAGGTAGGC	CACATCCATC	CCAAAGGAAA	ATGTAGACAT	500
CACCTGTACC	TATATAAGGA	TAAAGGCATG	TGTATAGAGC	AAAAAAAAAA	550
AAAAAAAAAA					560

## (2) INFORMATION FOR SEQ ID NO:24:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 1721  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

CTAGAGCTTT	CGACTCTCCG	CTGCGCGGCA	GCTGGCGGGG	GGAGCAGCCA	GGTGAGCCCA	60
AGATGCTGCT	GCGCTCGAAG	CCTGCGCTGC	CGCCGCGGCT	GATGCTGCTG	CTCCTGGGGC	120
CGCTGGGTCC	CCTCTCCCTT	GGCGCCCTGC	CCCGACCTGC	GCAAGCACAG	GACGTCGTGG	180
ACCTGGACTT	CTTCACCCAG	GAGCCGCTGC	ACCTGGTGAG	CCCCTCGTTC	CTGTCCGTCA	240
CCATTGACGC	CACCTTGGCC	ACGGACCCGC	GGTTCCTCAT	CCTCCTGGGT	TCTCCAAAGC	300
TTCTGACCTT	GGCCAGAGGC	TTGTCTCCTG	CGTACCTGAG	GTTTGGTGGC	ACCAAGACAG	360
ACTTCCTAAT	TTTCGATCCC	AAGAAGGAAT	CAACCTTTGA	AGAGAGAAAT	TACTGGCAAT	420
CTCAAGTCAA	CCAGGATATT	TGCAAAATATG	GATCCATCCC	TCCTGATGTG	GAGGAGAAGT	480
TACGGTTGGA	ATGGCCCTAC	CAGGAGCAAT	TGCTACTCCG	AGAACACTAC	CAGAAAAAGT	540
TCAAGAACAG	CACCTACTCA	AGAAGCTCTG	TAGATGTGCT	ATACACTTTT	GCAAACTGCT	600
CAGGACTGGA	CTTGATCTTT	GGCCTAAATG	CGTTATTAAG	AACAGCAGAT	TGCAAGTGA	660
ACAGTTCTAA	TGCTCAGTTG	CTCCTGGACT	ACTGCTCTTC	CAAGGGGTAT	AACATTTCTT	720
GGGAAGTAGG	CAATGAACCT	AACAGTTTCC	TTAAGAAGGC	TGATATTTTC	ATCAATGGGT	780
CGCAGTTAGG	AGAAGATTAT	ATTCAATTGC	ATAAACTTCT	AAGAAAGTCC	ACCTTCAAAA	840
ATGCAAAACT	CTATGGTCTT	GATGTTGGTC	AGCCTCGAAG	AAAGACGGCT	AAGATGCTGA	900
AGAGCTTCTT	GAAGGCTGGT	GGAGAAGTGA	TTGATTCAGT	TACATGGCAT	CACTACTATT	960
TGAATGGACG	GACTGCTACC	AGGGAAGATT	TTCTAAACCC	TGATGTATTG	GACATTTTAA	1020
TTTCATCTGT	GCAAAAAGTT	TTCCAGGTGG	TTGAGAGCAC	CAGGCCTGGC	AAGAAGGTCT	1080
GGTTAGGAGA	AACAAGCTCT	GCATATGGAG	GCGGAGCGCC	CTTGCTATCC	GACACCTTTG	1140
CAGCTGGCTT	TATGTGGCTG	GATAAATTGG	GCCTGTCAGC	CCGAATGGGA	ATAGAAGTGG	1200
TGATGAGGCA	AGTATTCTTT	GGAGCAGGAA	ACTACCATT	AGTGGATGAA	AACCTCGATC	1260
CTTTACCTGA	TTATTGGCTA	TCTCTTCTGT	TCAAGAAATT	GGTGGGCACC	AAGGTGTTAA	1320
TGGCAAGCGT	GCAAGGTTCA	AAGAGAAGGA	AGCTTCGAGT	ATACCTTCAT	TGCACAAACA	1380
CTGACATCC	AAGGTATAAA	GAAGGAGATT	TAACCTGTGA	TGCCATAAAC	CTCCATAACG	1440
TCACCAAGTA	CTTGCGGTAA	CCCTATCCTT	TTTCTAACAA	GCAAGTGGAT	AAATACCTTC	1500
TAAAGACCTT	GGGACCTCAT	GGATTACTTT	CCAAATCTGT	CCAACCTCAAT	GGTCTAACTC	1560
TAAAGATGGT	GGATGATCAA	ACCTTGCCAC	CTTTAATGGA	AAAACCTCTC	CGGCCAGGAA	1620
GTTCACTGGG	CTTGCCAGCT	TTCTCATATA	GTTTTTTTGT	GATAAGAAAT	GCCAAAGTTG	1680
CTGCTTGCA	CTGAAAATAA	AATATACTAG	TCCTGACACT	G		1721

## (2) INFORMATION FOR SEQ ID NO:25:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 45  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:  
CTTACTTGTG ATCGTGTGCC TTGTAGTCTC GGTAGCGGCA GGCCA 45